Natural history of meal-induced changes in blood pressure, gastric emptying and incretin hormone secretion and approaches to the management of postprandial hypotension

Thesis by

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TABLE OF CONTENTS

THESIS SUMMARY

This thesis presents a series of clinical research studies focusing on postprandial blood pressure (BP), glycaemic and incretin hormone responses in healthy ageing. The studies address the underlying pathophysiology, natural history, and approaches to management, of postprandial hypotension (PPH), longitudinal changes in and relationship between postprandial plasma levels of the incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP).

PPH, usually defined as a fall in systolic BP of greater than, or equal to, 20 mmHg within 2 h of a meal occurs frequently, particularly in older people and those with type 2 diabetes (T2D). PPH increases the incidence of falls, syncope, cerebrovascular disease, angina, and has been associated with a higher risk of cardiovascular mortality even when it is asymptomatic. After a meal, there is a substantial increase in splanchnic blood flow, leading to a reduction of blood volume returning to the heart. PPH occurs when compensatory responses are inadequate to maintain BP. Multiple factors are involved in the pathophysiology of PPH, including small intestinal nutrient delivery, changes in autonomic function, the release of gastrointestinal hormones and changes in splanchnic blood flow. **Chapter 1, 2 and 3** are structured as narrative reviews which aim to provide a comprehensive background to the studies. **Chapter 1** summarises 'up-to-date' knowledge relating to PPH, including the prevalence, clinical relevance, pathophysiology and approaches to management. **Chapter 2** is a brief review of the physiology of gastric emptying, which is pivotal to the pathophysiology of PPH, while **Chapter 3** focusses on the interrelated relationship between gastric emptying and glycaemia.

In healthy older subjects and patients with T2D, there is a correlation between the magnitude of fall in BP induced by glucose with the rate of gastric emptying of glucose, so that relatively

more rapid gastric emptying is associated with a greater fall in BP. Cross-sectional studies indicate that healthy ageing is associated with a modest slowing of gastric emptying, however, there is limited information about longitudinal changes in gastric emptying in a healthy, ageing population and no studies which have evaluated the natural history of the fall in BP induced by glucose with ageing. In the study described in **Chapter 4**, longitudinal changes in the BP response to, and gastric emptying of, glucose were evaluated in 33 healthy older people at an initial study and after 5.8 ± 0.1yr. BP, heart rate (HR) and gastric emptying (using a stable isotope breath test technique) were assessed concurrently after participants consumed a 300mL drink containing 75g glucose and 150mg C13-acetate.

PPH is under-recognised, but common. Following health concerns about excessive consumption of sugar, there has been an increasing trend to use low- or non-nutritive sweeteners as an alternative. Due to the lack of literature in this area, a systematic review described in **Chapter 5** was conducted to identify important gaps in information relevant to the effects of different types of sweeteners on postprandial BP.

While all macronutrients reduce BP comparably, the hypotensive responses to fat and protein occur slightly later than the response to glucose in healthy older people, probably reflecting the more prolonged time for digestion. Moreover, xylose, a poorly absorbed pentose sugar, empties from the stomach at a comparable rate to glucose, but has no effect on BP in healthy older subjects, as is also the case for fructose. The effect of artificial sweeteners, such as sucralose, on postprandial BP, was unknown. In the study described in **Chapter 6**, the effects of intraduodenal (ID) infusion of sucralose and glucose versus saline, on BP and HR, superior mesenteric artery (SMA) blood flow and blood glucose, were assessed in healthy older individuals.

Current management of PPH is suboptimal. Acarbose is known to attenuate the fall in systolic BP induced by oral sucrose in healthy older adults, associated with slowing of gastric emptying and enhanced release of GLP-1. Gastric distension with water at a volume as low as 300 mL mitigates the fall in BP in response to ID glucose. In the study described in **Chapter 7**, the effects of gastric distension and acarbose, either alone or in combination, on BP, glycaemia and SMA flow after oral sucrose were assessed in healthy older people.

A whey protein/guar preload has been shown to reduce postprandial glycaemia in T2D, an effect suggested to be mediated by slowing of gastric emptying. However, the latter has only been assessed using a stable isotope breath test technique, which cannot discriminate between slowing of gastric emptying and a delay in small intestinal absorption. This preload also has potential for use in the management of postprandial hypotension. In the study reported in **Chapter 8**, the effects of a guar/whey protein preload on gastric emptying (using scintigraphy), glucose absorption, glycaemic/insulinaemic and BP responses to an oral glucose load, were evaluated in healthy older people.

The rate of gastric emptying is a major determinant of the glycaemic response to carbohydrate-containing meals in healthy subjects, as well as individuals with T2D. Gastric emptying also influences the release of incretin hormones, GLP-1 and GIP, which impact postprandial glycaemic excursions. It is not known whether baseline and/or nutrient-stimulated GLP-1 or GIP levels are predictable within an individual or affected by ageing. The study described in **Chapter 9** re-evaluated a cohort of healthy older subjects after an interval of ~ 5.9 years and determined changes in fasting and glucose-stimulated plasma GLP-1 and GIP concentrations and their relationships with gastric emptying.

The incretin hormones, GLP-1 and GIP, are secreted following intestinal macronutrient exposure - GIP primarily from the proximal small intestine and GLP-1 from the more distal small intestine and colon. Their relative importance to the incretin effect in health has been contentious, although recent studies employing a specific GIP antagonist now indicate that GIP has the dominant role. It is uncertain whether there is a relationship between GIP and GLP-1 secretion. The study described in **Chapter 10** evaluates the relationship between GIP and GLP-1 responses to a 75g oral glucose load in older individuals with either normal (NGT) or impaired glucose tolerance (IGT).

DECLARATION

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission in my name, for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

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PUBLICATIONS ARISING FROM THESIS

1. Pham HT, Stevens JE, Rigda RS, Phillips LK, Wu T, Hausken T, Soenen S, Visvanathan R, Rayner CK, Horowitz M, Jones KL. **Effects of intraduodenal administration of the artificial sweetener, sucralose, on blood pressure and superior mesenteric artery blood flow in healthy older subjects**. Am J Clin Nutr. Jul 2018;108(1):156-162. doi: 10.1093/ajcn/nqy060.

2. Pham HT, Phillips LK, Trahair LG, Hatzinikolas S, Horowitz M, Jones KL. **Longitudinal changes in the blood pressure responses to and gastric emptying of, an oral glucose load in healthy older subjects**. J Gerontol A Biol Sci Med Sci 2019. doi: 10.1093/gerona/glz014.

3. Pham HT, Phillips LK and Jones KL, **Acute Effects of Nutritive and Non-Nutritive Sweeteners on Postprandial Blood Pressure**. Nutrients, 2019. 11(8).

4. Pham HT, Marathe CS, Phillips LK, Trahair LG, Hatzinikolas S, Huynh L, Wu T, Nauck MA, Rayner CK, Horowitz M, Jones KL. **Longitudinal changes in fasting and glucose-stimulated GLP-1 and GIP in healthy older subjects**. J Clin Endocrinol Metab. 2019 Aug 8. pii: jc.2019-01262. doi: 10.1210/jc.2019-01262.

5. Pham HT, Trahair LG, Phillips LK, Rayner CK, Horowitz M, Jones KL. **A randomized, crossover study of the acute effects of acarbose and gastric distension, alone and combined, on postprandial blood pressure in healthy older adults**. BMC Geriatr. 2019 Aug 30;19(1):241. doi: 10.1186/s12877-019-1251-7.

6. *Marathe CS, *Pham HT, Marathe JA, Trahair LG, Huynh L, Wu T, Phillips LK, Rayner CK, Nauck MA, Horowitz M, Jones KL. **Relationships between GIP and GLP-1 secretion in response to an oral glucose load in participants with normal and impaired glucose tolerance**. Acta Diabetologica (*Accepted for Publication*). **Joint first author*

7. Pham HT, Holen I, Phillips LK, Hatzinikolas S, Huynh L, Wu T, Rayner CK, Horowitz M, Jones KL. **Effects of a guar and whey containing preload (Omniblend) on gastric emptying, glycaemia, small intestinal absorption and blood pressure responses to oral glucose in healthy older subjects.** Nutrients. 2019 Nov 5;11(11). pii: E2666. doi: 10.3390/nu11112666.

Chapter 1

Postprandial hypotension

1.1 Introduction

Postprandial hypotension (PPH) appears to be, an important clinical condition, which is certainly under-recognised. PPH may result in an increased incidence of falls, syncope, cerebrovascular disease, angina, and is apparently associated with a higher risk of cardiovascular mortality [1, 2] even when it is asymptomatic [3]. The clinical significance of PPH will, however, remain controversial in the absence of intervention studies demonstrating benefit. The latter, by definition, requires an effective and safe intervention which is currently lacking. PPH is generally defined as a fall in systolic blood pressure (BP) of greater than or equal to 20 mmHg, or a postprandial systolic BP less than or equal to 90 mmHg when the pre-prandial systolic BP is greater than or equal to 100 mmHg, within 2 h of a meal [1].

PPH is distinct from orthostatic hypotension and prevalence increases with age [4]. PPH occurs frequently in healthy older subjects (13-38%) [5] and patients with autonomic dysfunction, often secondary to type 2 diabetes (T2D) (~35%) [1, 5] or Parkinson's disease (40-100%) [1, 5]. The 'early' dumping syndrome, which is reported in approximately 25–50% of patients after gastric surgery including Roux-en-Y gastric bypass for management of obesity, also includes PPH [4, 6].

The pathophysiology of PPH is incompletely understood, and a number of factors play a role in the fall in BP, including small intestinal nutrient delivery [7, 8], changes in splanchnic blood flow [9] and neural and hormonal mechanisms [4], while gastric distension attenuates the fall in postprandial BP [10, 11]. After a meal, there is a substantial increase in splanchnic blood flow, leading to a reduction of blood volume returning to the heart [1]. In healthy young and non-PPH older individuals with intact baroreflex mechanisms, the reduced blood volume in the systemic circulation is compensated by concomitant increases in heart rate (HR), stroke

volume and cardiac output to maintain BP [12]. PPH reflects the inadequacy of these protective responses [1].

Current strategies for the management of PPH include nonpharmacologic and pharmacologic approaches. Macronutrient modification [13], consumption of smaller and more frequent meals [13], maintenance of adequate fluid intake [1], drinking water before a meal [11, 14, 15], and exercise after a meal [16] are non-pharmacological approaches which may attenuate the decrease in BP, while caffeine [17], acarbose [18], and octreotide [19, 20], variations in the timing and doses of antihypertensive drugs [21] are the most common pharmacological approaches. While both nonpharmacologic and pharmacologic treatments can have efficacy, in general, they appear limited and none is targeted at the underlying pathophysiology. More recently, there is evidence that interventions based on slowing gastric emptying [22, 23] and small intestinal absorption of nutrients [24], while maximising gastric distension are effective in attenuating PPH. Exogenous glucagon-like peptide-1 (GLP-1) and GLP-1 receptor agonists have received considerable attention in the last few years as a potential novel therapeutic approach to the management of PPH.

This chapter provides current knowledge relating to PPH, with a particular focus on prevalence, pathophysiology, and management, including future priorities for research.

1.2 Prevalence of PPH

The majority of individuals with PPH either are old or have diabetes and/or a neurological disorder.

1.2.1 Older people

PPH was reported to occur in 118 out of 449 (24%) [25] and 41 out of 114 (36%) [26] elderly nursing home residents after a standardised carbohydrate meal in two cohort studies. A subsequent study demonstrated 57 out of 85 elderly hospitalised patients (67%) had PPH with a mean postprandial reduction in systolic BP of ~ 34 mmHg [27], while the prevalence of orthostatic hypotension was 52% [27]. Although they are distinct phenomena, there is a strong correlation between orthostatic hypotension and PPH, i.e. subjects with orthostatic hypotension are much more prone to PPH [28]. A cohort study conducted in 5888 older community-dwelling individuals found that systolic and diastolic BPs were lower (~ 130.1/66.6 mmHg) within the first hour after a non-standardised meal, compared to measurements immediately following the meal (~ 133.7/68.8 mmHg) and four hours after the meal (~ 136.5/71.1 mmHg) [29]. In 150 long-term care patients receiving a high carbohydrate meal via either oral, nasogastric infusion or percutaneous endoscopic gastrostomy feeding (50 in each group), PPH was evident in 64 (43%) patients and there was no difference in PPH prevalence among the three groups [30]; 9 out of 12 (75%) fed through the gastrostomy tube had PPH [31]. In older adults with a history of falls, the prevalence of PPH was 42.1 ± 5.1% after a test meal [32].

The postprandial fall in BP may be exaggerated in the setting of multiple comorbidities [26] and multiple-drug therapy [25]. More recently, PPH has been shown to be both prevalent and underdiagnosed in older survivors of the Intensive Care Unit (ICU). In a cohort of 35 older survivors (>65 years) discharged in the preceding 3 months from ICU, 10 (29%) had PPH [33]. Although PPH is widely appreciated as a common condition in healthy older subjects, there had been no studies investigating its natural history before the commencement of my PhD. This gap in the literature triggered my study which focused on the trajectory of the disorder (**Chapter 4**).

1.2.2 Diabetes

T2D and type 1 diabetes (T1D) are both associated with autonomic nerve dysfunction [34], which predisposes to PPH [35]. Sasaki et al. reported that among a cohort of 35 patients with T2D, PPH was identified in 37% after ad libitum eating and in 20% after the ingestion of a glucose load (75g in 300 mL water), and suggested that PPH may be an important cardiovascular disorder in T2D [36]. Subsequently, Jones et al. reported in a cohort of 16 T2D patients who ingested 75g glucose, the prevalence of PPH was 44% [7]. In these studies only a minority of subjects exhibited symptoms of PPH [7, 36], but Sasaki et al. suggested that PPH increased the risk of sudden death as well as myocardial and cerebral infarction [36]. More recently, Hashizume et al. have demonstrated a postprandial fall in both central and brachial systolic BP in patients with T2D – the decrease in BP was more prolonged in those with severe, compared with mild, cardiac autonomic dysfunction [35]. Although PPH has also been reported to occur in patients with T1D, the prevalence in this group is uncertain [37].

1.2.3 Neurological diseases

A recent meta-analysis confirmed that the likelihood of PPH is higher in patients with neurological diseases [38]. The mechanisms underlying PPH in neurological diseases have generally been attributed to autonomic failure which may be the primary and associated with an unknown aetiology (e.g., multiple system atrophy, synonymous with the Shy-Drager syndrome and Parkinson's disease) or secondary as associated with a clearly defined cause (e.g., spinal cord lesions, drugs and, as discussed, diabetes) [39]. In patients with autonomic failure, a major fall in systolic BP to around 80/50 mmHg, persisting for more than 3 hours in the supine position, has been reported [40, 41]. PPH has also been identified in several small cohorts of patients with autonomic failure [42-45]. Prevalence of PPH of 42.5 - 82% in patients

with Parkinson's disease has been reported [46-51]. In a recent study by Trahair et al. of 21 patients with mild to moderate Parkinson's disease (Hoehn and Yahr score 1.4 ± 1), 8 (38%) had PPH after a 75g oral glucose load and in a further 8 (38%), the fall in systolic BP was > 10 mmHg, but < 20 mmHg [52]. Idiaquez et al. [53] found that 70% of patients with severe Alzheimer's disease had a fall in BP of ≥ 20 mmHg after a standard meal. PPH also occurs in a high percentage (52.9-70%) of patients with multiple system atrophy [38, 54].

1.2.4 Other illnesses

Dumping syndrome is a frequent complication of gastric surgery associated with a drainage procedure. In the past, such surgery was usually performed for the management of peptic ulcer (e.g. Billroth 2 gastrectomy and vagotomy pyloroplasty); an increasingly common reason is Roux-en-Y gastric bypass (RYGB) surgery for the management of obesity. Features of dumping syndrome have been noted in more than 50% in the early postoperative stage of the Billroth II gastrojejunostomy [55]. Dumping syndrome can be divided into either 'early' or 'late' symptoms. Clinical manifestations of early dumping syndrome include gastrointestinal and systemic vasomotor symptoms, such as a postprandial fall in BP, with symptoms occurring within 30 min following meal ingestion [55, 56]. Late dumping syndrome occurs occur 1–3 h after food ingestion and consists of symptoms associated with hypoglycaemia [55, 56]. Dumping is associated with a decreased quality of life and increased morbidity [6]. Nguyen et al. reported that 2 out of 10 unselected patients after RYGB had the dumping symptoms including hypotension and dizziness, following a 50g glucose drink in the sitting position [57]. More recently, Takeshita et al. reported a case of severe early dumping syndrome associated with markedly increased splanchnic vasodilatation requiring urgent treatment with intravenous fluids and vasopressors [58].

There is a concern that eating during haemodialysis may exacerbate the fall in BP which occurs frequently [59-61]. Sherman et al. reported 13 events of symptomatic hypotension in five patients who were given a standard meal during dialysis, and two events in one fasting patient [62]. Subsequently, the magnitude and frequency of the fall in BP were greater after a standard snack (400 kcal) compared with control in 13 patients with chronic renal failure during haemodialysis [61]. Borzou et al. reported that food ingestion during haemodialysis induced a fall in systolic and diastolic blood pressures, which persisted for one hour (systolic) and one and a half-hours (diastolic) following the meal [59]. Given these findings, the prevalence and clinical relevance of postprandial hypotension in the dialysis setting need further investigation.

1.3 Significance of PPH

PPH is a clinically important condition associated with increased morbidity and mortality. It has been shown to be associated with an increased risk of falls. For example, Puisieux et al. reported a high percentage (23%) of PPH in a cohort of elderly patients hospitalised for either syncope or falls [63]. PPH has also been identified as a risk factor for both cerebrovascular [64-66] and cardiovascular events [3, 66, 67]. A relationship between evidence of asymptomatic cerebrovascular damage on magnetic resonance imaging (MRI) and the magnitude of the fall in postprandial BP has been demonstrated, i.e. the prevalence of lacunar infarction and advanced leukoaraiosis was greater in individuals with a fall in BP of > 10 mmHg (83% for both), compared to those in whom the fall in BP was < 10 mmHg (69% and 50% respectively) and the normal group (44% for both) [64]. A postprandial BP decline is also considered important in the aetiology of asymptomatic lacunar infarction in community dwellers [2]. A Japanese cross-sectional study was performed from February 2006 to August 2012 and involving 1308 apparently healthy, middle-aged to elderly, people who underwent brain MRI and postprandial BP measurements. Based on the postprandial change in systolic

BP (ΔSBP), participants were classified into four subgroups: ΔSBP≤ −20 mmHg, −20 < ΔSBP≤ −10 mmHg, −10 < ΔSBP ≤ 10 mmHg and ΔSBP > 10 mmHg. There was a significant difference in the prevalence of lacunar infarction among the four subgroups (12.7%, 11.4%, 8.1%, and 7.2% respectively, P = 0.007) [2]. Another study showed that over a 4-year period, a breakfast-induced fall in BP was associated with an increased risk of cardiovascular mortality in patients with PPH [67]. In a group of 179 elderly low-level-care nursing home residents, PPH appears to be an independent predictor of all-cause mortality [68]. Over a period of 4.7 years, the mortality rate in nursing home patients with PPH was greater than that in patients without PPH (145.0 vs. 98.5 per 1,000 person-years) [68]. Moreover, there was a strong correlation between the risk of death and the magnitude of the postprandial reduction in BP - patients who exhibited a postprandial reduction in BP ≥ 40 mmHg had the highest mortality (156.1 per 1000 person-years), followed by those with a postprandial reduction in BP of 20 - 39, 11–19 and ≤10 mmHg (144.4, 116.9 and 89.1 per 1000 person-years respectively) [68] (Figure 1.1).

1.4 Pathophysiology of PPH

1.4.1 Gastric emptying

In both health and diabetes, the rate of gastric emptying has a wide interindividual variation from 1 to 4 kcal/min, but minimal intraindividual variation [69, 70]. This range of gastric emptying is greater in T2D due to a high prevalence of disordered (slower and more rapid) emptying [71]. The rate of gastric emptying is determined primarily by the interaction between the motor activity of the fundus, antrum and pylorus, negative feedback arising from the interaction of nutrients with the receptors located in the small intestinal, and gastrointestinal hormones released in response to food ingestion [69, 72, 73].

In healthy older subjects [70, 74] and patients with T2D [7] (Figure 1.2), there is a correlation between the postprandial fall in BP with the rate of gastric emptying of glucose. The finding is supported by the outcomes of several other studies which have used intraduodenal (ID) nutrient infusion to 'bypass' the potential confounding effects of gastric distension and differences in gastric emptying rates between individuals [8, 24, 75, 76]. In healthy older subjects, the rate of small intestinal delivery must reach a specific threshold to result in hypotensive effects. O'Donovan et al. reported that 3 kcal/min ID glucose infusion induced a substantial decrease in BP and rise in HR in healthy elderly subjects whereas a 1 kcal/min infusion had no effect [8]. Similarly, in a more recent study, a 2 kcal/min intraduodenal glucose infusion lead to a comparable reduction in BP to a 3 kcal/min infusion, but a 1 kcal/min had no effect [24] (Figure 1.3). In a recent study involving 9 patients with T2D, the fall in systolic BP was greater when ID glucose infusion was increased from 2 kcal/min to 4 kcal/min [76]. Furthermore, dietary interventions such as the viscous polysaccharide guar which retards gastric emptying [22, 23] and small intestinal absorption of nutrients [24] attenuate the fall in BP effectively.

1.4.2 Autonomic and Neural Mechanisms

Food ingestion is followed by a redistribution of blood into the splanchnic vessels to optimise digestion and absorption of nutrients [77]. In healthy, young subjects, the so-called ''gastrovascular reflex'' is associated with postprandial increases in muscle sympathetic nerve activity (MSNA), HR, cardiac output and, accordingly BP [1, 63, 78-81]. These responses are associated with an elevation of plasma noradrenaline [1]. While similar effects occur in healthy older people, albeit to a lesser degree than in young healthy controls [79, 82], the increases in the superior mesenteric artery (SMA) flow does not differ between the two groups [83]. Accordingly, to stabilise postprandial BP, sympathetic nerve activity must increase to more

than 200% of basal values in healthy older people [84]. Hypotension reflects an imbalance between the increase in splanchnic blood flow and inadequate compensatory responses [84-87].

A comparable increase in MSNA is also observed in healthy young and older subjects in response to ID glucose [88]. That healthy elderly subjects exhibit a fall in BP, is suggestive of decreased sympathetic baroreflex sensitivity [88].

Gastric distension increases MSNA [89], but has no effect on plasma noradrenaline in the healthy elderly [90]. Vanis et al. [90] reported an increase in plasma norepinephrine concentrations following an ID glucose load (3 kcal/min) during intragastric balloon distensions of 0, 100, 300, or 500 mL, with no difference in plasma concentrations between distensions. In this study, a fall in systolic BP was observed only during the 0 mL intragastric distension [90]. Another study reported that MSNA could be increased by progressive gastric distension (intragastric bag) in both young and older healthy subjects and that the increases were greater in the former group [89].

Nitric oxide, an important neurotransmitter in the gastrointestinal tract, mediates vasodilation of blood vessels in both animals [91, 92]and humans [93]. Specific inhibitors of its production, such as NG-mono-methyl-L-arginine (L-NMMA) and NG-nitro-L-arginine-methyl-ester (L-NAME) can be used to address the role of nitric oxide [91, 94] and Gentilcore et al. reported, in healthy older subjects, that acute administration of NG-nitro-L-arginine-methyl-ester decreased the hypotensive response to oral glucose, but had no effect on gastric emptying [95].

1.4.3 Hormonal Mechanisms

Although there is no conclusive evidence of an important role for hormonal mechanisms in PPH, a number of gastrointestinal peptides, including insulin, GLP-1, glucagon-like peptide-2 (GLP-2), glucose-dependent insulinotropic peptide (GIP), calcitonin-gene-related peptide (CGRP), neurotensin, vasoactive intestinal peptide (VIP), bradykinin, and substance P, may be involved in postprandial BP regulation [1, 96]. Administration of somatostatin and its analogues, which suppress the release of most gastrointestinal hormones, have been shown to decrease the postprandial fall in BP [19, 45], although somatostatin may also affect splanchnic blood flow directly [97].

1.4.3.1 Insulin

Several early studies [98-100] supported the role of insulin in modulating PPH through its known vasodilatory effects [101], and capacity to modulate sympathetic function [12]. However, it is unlikely that insulin plays a primary role in PPH. Intravenous administration of glucose [102], which is a substantial stimulus to insulin secretion, has no effect on BP and there is a postprandial fall in BP in people who have T1D [37], which is associated with absent endogenous insulin secretion. It is also reported that neither fructose nor xylose [103, 104], unlike glucose, when administered in an isosmotic solution has an influence on BP.

1.4.3.2 Glucagon-like peptide-1

GLP-1 is produced in L-cells in the distal small intestine in response to the exposure to nutrients and bile acids [105]. As will be discussed in **Chapter 3**, the main function of GLP-1 is to act as an incretin hormone by stimulating insulin secretion and inhibiting glucagon secretion, both

in a glucose-dependent manner. GLP-1 also modulates gastrointestinal motility and slows gastric emptying [106]. Endogenous GLP-1 may play a role to reduce the postprandial fall in BP. The effect of acarbose to attenuate the fall in BP induced by oral [18] or intraduodenal [107] sucrose in healthy older subjects is associated with the stimulation of GLP-1 [18, 108]. In healthy older subjects, the postprandial fall in BP after ID or oral ingestion of glucose is also attenuated by intravenous GLP-1 in healthy older subjects [106, 109]. Furthermore, the dipeptidyl peptidase 4 (DPP-4) inhibitor, vildagliptin, which stimulates GLP-1 was reported to be effective in treating an 85-year-old woman with symptomatic PPH [110]. More recently, acute administration of the GLP-1 receptor agonists, lixisenatide, has been shown to prevent the fall in BP induced by glucose in healthy older subjects and T2D patients [111]. This study is described in more detail in the "management" section.

1.4.3.3 Glucagon-like peptide-2

GLP-2, which is co-secreted with GLP-1 from L-cells with the ratio 1:1 [112], increases mesenteric blood flow [113-115] and may contribute to the pathogenesis of PPH [96]. There is, however, little information relating to the relevance of GLP-2 to PPH. Fukushima et al. [96] reported no difference in postprandial plasma GLP-2 concentrations in multiple system atrophy patients with and without PPH. In animal models, GLP-2 reduces antral contractility and fundic tone [112] but does not appear to affect gastric emptying in humans [112].

1.4.3.4 Glucose-dependent insulinotropic peptide

GIP is secreted from K-cells, which are located primarily in the proximal small intestine [116]. As will be discussed in **Chapter 3**, GIP stimulates the release of insulin and glucagon from β and α cells in a glucose-dependent fashion [117]. Unlike GLP-1, GIP has little or no effect on

gastric emptying [118]. Several animal studies have demonstrated that after food intake, GIP secretion potentiates the release of GLP-1 [119] but this is not the case in humans [119] - a direct role for GIP in postprandial BP regulation has hitherto not been evaluated due to the absence of a GIP antagonist.

1.4.3.5 Vasoactive intestinal polypeptide, substance P, calcitonin gene-related peptide

VIP is both a neuromodulator and neurotransmitter [120, 121]. Its primary functions are to regulate smooth muscle activity, epithelial cell secretion, and blood flow in the gastrointestinal tract. Because of its vasodilatory properties, it might be relevant to the pathogenesis of PPH, although there is no difference between pre- and postprandial plasma levels of VIP in patients with autonomic neuropathy or in older subjects [12]. Furthermore, plasma concentrations of VIP are not affected by the administration of octreotide, which markedly attenuates the postprandial fall in BP [20].

Substance P, like VIP, probably functions more as a neurotransmitter than as a circulating vasoactive hormone [122] and does not appear to have a role in PPH [102]. Ingestion of glucose or a meal has no effect on plasma substance P concentrations in either patients with autonomic failure or healthy older subjects [20, 101, 123].

CGRP is a neuropeptide with vasodilatory effects. Edwards et al. have reported a significant relationship ($R = -0.37$, $P = < 0.05$) between the increase in CGRP and the change in BP after a 75 g glucose load in healthy older subjects [124] and in the group of subjects who exhibited a fall in BP > 15 mmHg, the increase in CGRP was greater in older subjects than in young and middle-aged subjects [124]. Further studies are warranted to define the role of CGRP in the hypotensive response to meals.

1.4.3.6 Neurotensin

Neurotensin, a potent vasodilatory peptide may increase intestinal blood flow after a meal [125, 126]. There is an increase in SMA blood flow after intravenous administration of neurotensin [102]. Observations relating to the impact of neurotensin on postprandial BP are, however, inconsistent [102]. In comparison with healthy individuals, plasma neurotensin levels increase more markedly after oral glucose in patients with autonomic neuropathy and PPH [127]. In contrast, Hoeldtke et al. found no change in plasma neurotensin after breakfast in patients with PPH [20], while Fukushima et al. reported that the plasma neurotensin levels increased comparably in patients with multiple system atrophy with or without PPH after a meal [96].

1.4.4 Meal Composition

Of the macronutrients, carbohydrate appears to have the greatest and most rapid suppressive effect on BP in healthy people and those with PPH [12, 40, 128]. Observations relating to the effects of fat and protein on postprandial BP have been inconsistent. While some studies have found that fat [128, 129] and protein [130] result in comparable postprandial falls in BP to glucose, other studies have reported that there is no change in BP after fat [12, 130, 131] and protein [12, 131] meals. Gentilcore et al. reported that glucose, fat, and protein evoke comparable falls in systolic BP after being infused intraduodenally, but the onset of the effect of glucose is earlier [9]. This may be because the effects of fat and protein on BP are dependent on their digestions to amino acids and free fatty acids [9].

A simple carbohydrate meal may induce a greater hypotensive effect than an isocaloric complex carbohydrate meal in healthy older people, probably reflecting the different

absorption rates in small intestine [132]. While both glucose and sucrose trigger comparable postprandial falls in BP in healthy older subjects, albeit with a delayed effect for sucrose [133], it is clear that fructose [133, 134] has no effect on BP and xylose [40, 103] only causes a modest fall in BP. These outcomes suggest that the hypotensive effect of carbohydrates is probably dependent on digestion to monosaccharides and their affinity for glucose transporters [133, 134]. Accordingly, sucrose, a disaccharide, must be converted to glucose and fructose for its hypotensive effect [133].

1.4.5 Superior Mesenteric Artery blood flow

After a meal, there is an approximately twofold increase in SMA blood flow, associated with decreases in systemic vascular resistance and skeletal muscle blood flow [1, 135, 136]. Sidery et al suggested that the increase in splanchnic flow is likely to be implicated in the fall in BP in healthy older subjects [129]. In favour of this argument, octreotide, which is known to suppress the release of gut hormones, markedly decreases the postprandial increase in SMA blood flow, and attenuates the postprandial fall in BP in autonomic failure patients [137]. However, the increase in mesenteric flow after a high carbohydrate meal in healthy young and older subjects is comparable [129], suggesting that PPH reflects, in the broadest sense, inadequate cardiovascular compensation for the increase in SMA blood [129, 135].

Many studies indicate that factors such as the size and composition of the meal, and the rate of small intestinal nutrient delivery affect the magnitude of the postprandial increase in mesenteric blood flow. A large meal might induce a greater postprandial hormonal response, leading to increased mesenteric vasodilatation [13]. The latter has been shown to be dependent on the macronutrient content [138] so that the increase in mesenteric blood flow is highest after glucose ingestion, followed by, in the absence of bile salts, fat and then protein. In contrast,

when bile salts are present, several long-chain fatty acids appear to have more potent hyperaemic effects than glucose [138]. In healthy older subjects, ID infusion of glucose has a greater, and earlier, effect on the increase in SMA blood flow, compared with fat or protein, and protein induces a lesser SMA blood flow than fat [9] (Figure 1.4). The rate of small intestinal nutrient delivery also influences SMA flow [24]. For example, 3 kcal/min ID glucose infusion triggers a greater increase in SMA blood flow, compared to 1 kcal/min and 2 kcal/min infusions [24].

1.4.6 Gastric distension

Gastric distension attenuates the postprandial fall in BP probably by triggering the 'gastrovascular reflex' [89]. ID glucose infusion (75g glucose/300 mL) induces a greater fall in BP in comparison to an equivalent oral glucose load [139] (Figure 1.5).

There is little information regarding the role of specific regions of the stomach in postprandial BP, however, there is some evidence that the proximal stomach may be more important [10, 89, 140]. Proximal gastric distension created with a so-called 'barostat' triggers 'gastrovascular reflex' leading to increases in BP, HR and MSNA in healthy young and older subjects [10, 89]. However, the gastrovascular reflex is attenuated in the latter group [89]. Jones et al. have reported relationships between increases in systolic BP with the volumes of the whole or proximal, but not the distal, stomach [140] and the magnitude of the 'gastrovascular reflex' is proportional to the gastric distension volume [140]. In the same way, drinking water has been shown to attenuate the postprandial fall in BP in healthy older subjects [141, 142], as well as patients with multiple system atrophy and pure autonomic failure [141-143]. Gentilcore et al. also reported that even a low volume of water (300 mL) infused intragastrically diminishes the fall in BP induced by ID glucose in the healthy elderly [15]. Interestingly, patients with

autonomic failure appear to have a greater pressor response to drinking water, compared to control subjects [142]. In a recent study by Trahair et al., the acute pressor response to water was preserved and, possibly exaggerated in patients with PPH [144].

1.5 Management

A standardised approach to the management of PPH remains to be established. Current strategies are both non-pharmacologic and pharmacologic - none is particularly effective. The majority of studies relating to the management of PPH have focussed on patients with autonomic failure without PPH, and asymptomatic healthy older people [145]. There is a major need for a safe, and clinically effective, targeted therapeutic strategy.

1.5.1 Non-pharmacologic

1.5.1.1 Dietary modifications

Meal frequency

It has been suggested that eating small meals frequently and reducing the carbohydrate portion of meals may attenuate postprandial falls in BP [13, 146]. A larger meal was shown to induce a greater increase in cardiac output, and greater decreases in peripheral vascular resistance and mean arterial pressure (MAP) in four healthy younger individuals, when compared to a smaller meal [146]. Consistent with this, Puvi-Rajasingham et al. reported that postprandial BP was lower in all positions (standing, sitting and lying) after consuming three large meals compared with six smaller meals with identical caloric intake in seven subjects with primary autonomic failure (Figure 1.6) [13]. Increasing the carbohydrate load was reported to induce greater postprandial falls in systolic and diastolic BP and exacerbate symptoms in elderly patients with

PPH [147]. Avoiding simple carbohydrate and consuming small-volume meals is also advised in patients with dumping syndrome [57, 148].

Glucose substitutes

As discussed, glucose is the most potent of the monosaccharides and more potent than fat and protein in inducing a fall in BP. The replacement of glucose with low nutritive sweeteners as well as non-nutritive sweeteners might represent a simple, and effective, way in preventing PPH. This issue represents the focus of the study reported in **Chapter 6** and the systematic review reported in **Chapter 5**.

Protein intake

Watson et al. have shown that short-term consumption (12 weeks) of a small amount of protein before meals twice daily in patients with well-controlled T2D reduces postprandial blood glucose by slowing gastric emptying [149], a strategy which may be applicable to the management of PPH. In a pilot study, high protein meals had no effects on symptoms of hypotension in patients undergoing haemodialysis [150]. The study reported in **Chapter 8** evaluates the effect of a protein 'preload' on postprandial BP in healthy older subjects.

1.5.1.2 Fluid intake

Intuitively, maintenance of intravascular volume in subjects predisposed to a postprandial fall in BP is important [1]. Ensuring adequate fluid and salt intake may facilitate the maintenance of intravascular volume and, therefore, minimise postprandial hypotension and its symptoms [151]. Similarly, it may be appropriate to withdraw or reduce diuretic therapy [152, 153]. Frusemide has been shown to increase the risk of PPH and is best-avoided [154].

1.5.1.3 Slowing of gastric emptying and small intestinal nutrient exposure

Guar gum, a gel-forming, unpalatable, and unabsorbable carbohydrate, derived primarily from the ground endosperm of guar beans and used as a bulking agent, has been shown to attenuate the postprandial fall in BP in healthy older subjects [22] and patients with T2D [155] when administered with a 50g glucose drink. The effects of guar on BP may be attributable to a number of mechanisms, including slowing of gastric emptying by increasing the viscosity of the intragastric content, and delaying small intestinal nutrient absorption by acting as a physical barrier between glucose and the small intestinal mucosal cells [22, 155]. Jang et al. have reported that postprandial systolic BP is greater, and the prevalence of PPH is less, after ingestion of a semi-fluid (rice (210g), soup (100g) and side dishes (70g)) with 9g guar, compared with control [156]. Consistent with these observations, an ID infusion of glucose (3 kcal/min) and guar (4g) was associated with a lesser fall in BP in comparison to a glucose infusion alone in healthy older subjects [23]. The potential use of guar in the management of PPH, unfortunately, is compromised markedly by its poor palatability and frequent gastrointestinal adverse effects including diarrhoea and flatulence.

As discussed, gastric distension has the potential to represent a simple and effective treatment for PPH [11, 140, 142, 157]. In patients with autonomic failure and in elderly people, drinking water prior to a meal attenuates the postprandial decrease in BP [11, 140-142]. Shannon et al. demonstrated that drinking water even in volume as low as 120 mL induced a substantial pressor effect in patients with primary autonomic failure [11]. Furthermore, in this study, systolic BP was substantially higher when the volume of water was increased to 480 mL [11]. Consistent with this, Jones et al. demonstrated that the fall in systolic and diastolic BP is greater during the first 60 min when glucose is given in a smaller volume (200 mL vs 600 mL) in healthy older subjects [140]. (Figure 1.7), while Gentilcore et al. reported that gastric distension

by intragastric infusion of water (300 mL) substantially inhibits the hypotensive response to the con-current ID glucose infusion in healthy elderly [15]. The effects of gastric distension are addressed further in **Chapter 7**.

1.5.1.4 Exercise

The effect of postprandial exercise on PPH appears limited. Oberman et al. found that a 20-minute walk after a meal had a pressor effect in frail older people [16]. However, this effect was transient and existent during the exercise [16]. More recently, 2 other studies have reported that intermittent walking attenuates the hypotensive response to a 50g glucose load in patients with PPH [158, 159]. Further studies are indicated.

1.5.2 Pharmacological

1.5.2.1 Caffeine

Caffeine suppresses adenosine-mediated splanchnic vasodilatation, and has accordingly, been suggested as a treatment for symptomatic patients [160, 161]. While there is evidence that caffeine may attenuate the postprandial fall in BP in healthy older subjects and patients with autonomic neuropathy when it is given immediately before or after a meal [160, 161], these studies were conducted in small cohorts ($n \leqslant 6$). In contrast, Lipsitz et al found that ingesting 250 mg caffeine during a meal failed to decrease the fall in systolic BP in autonomic failure patients with symptomatic PPH [17]. Therefore, the efficacy of caffeine in PPH management is uncertain.

1.5.2.2 α-glucosidase inhibitors

α-glucosidase inhibitors such as acarbose, voglibose and miglitol are often used in the treatment of T2D to lower blood glucose. Their suppressive effect on postprandial hyperglycaemia without the risk of hypoglycaemia has been attributed to slowing gastric emptying [18, 162, 163] and small intestinal absorption of carbohydrate [164, 165]. More recently, these drugs have been found to attenuate the fall in postprandial BP in healthy older people, patients with T2D and in those with neurologic disorders [18, 126, 166, 167], possibly in part, by inhibiting the release of vasodilatory peptides [126, 166] and stimulating the release of GLP-1 [18, 162, 163, 168-170]. A patient with T1D and severe PPH symptoms was reportedly treated successfully with acarbose [37]. Interestingly, Gentilcore et al. reported that when the protective factor of gastric distension is 'bypassed' by infusing sucrose directly into the duodenum, acarbose still attenuates the rise in splanchnic blood flow and the postprandial fall in BP [107]. α-glucosidase inhibitors are associated with a high prevalence of gastrointestinal adverse effects which may compromise their use [107, 167], however, they appear promising in pharmacologic management for PPH. Moreover, acarbose has the potential to have additive or synergistic effects to those of gastric distension. This use is addressed in the study reported in **Chapter 7**.

1.5.2.3 Somatostatin analogues

Octreotide, a somatostatin analogue, attenuates the postprandial fall in BP in healthy older people, patients with autonomic failure and hypertension [19, 45, 171, 172]. Its anti-hypotensive effect probably reflects suppression of the secretion of vasoactive gastrointestinal hormones and/or insulin and increasing splanchnic and peripheral vascular resistance [19, 171]. In a seminal study, Jansen et al. showed that a single dose of subcutaneous octreotide (50

micrograms) completely abolished the postprandial decrease in systolic BP in healthy older people and hypertensive patients following an oral glucose load [19]. In another study involving 18 patients with primary autonomic failure, both systolic and diastolic BPs were greater after octreotide compared with control [172]. Octreotide has also been shown to delay gastric emptying in healthy subjects [173-175]. Although octreotide has been proven promising in PPH treatment, its use is compromised by its expense, the inconvenience of daily injections and a high prevalence of adverse effects [20, 45]. Lanreotide, a long-acting somatostatin analogue, reportedly reduces the meal-induced splanchnic hyperaemia in healthy subjects [176], and has the potential to improve both compliance and patient quality of life [177]. However, there have been no studies focusing on its effect on postprandial BP.

1.5.2.4 GLP-1 receptor agonists

GLP-1 receptor agonists (GLP-1RAs) are now widely used in the management of T2D. 'Short-acting' GLP-1RAs (exenatide BID and lixisenatide) reduce postprandial glycaemic excursions markedly, primarily as a result of slowing gastric emptying [178].

In a recent study by our group [111], both healthy (n = 15) and T2D (n = 15) participants were given an acute subcutaneous administration of either 10mcg lixisenatide or matching placebo 30 min before a 75g glucose drink. This study was stimulated by the positive effects of intravenous GLP-1 to reduce the hypotensive response to oral [109] and ID [106] glucose. Lixisenatide markedly slowed gastric emptying of the glucose drink [111, 179] (Figure 1.8), but also attenuated the increase in SMA flow and prevented glucose-induced fall in BP. In T2D, intravenous exenatide has been reported to attenuate the fall in BP during an ID glucose infusion, presumably via slowing of small intestinal absorption and transit [180]. These studies have established 'proof-of-principle' for the use of 'short-acting' GLP-1 RAs in the

management of PPH. These drugs are relatively expensive, but in general, well-tolerated: further studies are required.

1.5.2.5 Metformin

Metformin, the oldest and most widely used glucose-lowering drug, is the 'first-line' medication for the treatment of T2D [181]. Metformin may have a preventive effect on cardiovascular disease [182]. A meta-analysis reported that in patients without diabetes, metformin is able to decrease systolic, but not diastolic, BP [183]. The effect of metformin on BP in T2D patients is inconsistent. Some early studies, in T2D patients with hypertension, reported a slight decrease in systolic BP after metformin [184, 185], however, this was not the case in all studies [186, 187]. In contrast, Borg et al. recently reported that compared with saline control, an acute dose of metformin (1g) administered intravenously 60 minutes before a 50g glucose in subjects with well-controlled T2D prevented any fall in SBP, slowed gastric emptying and increased plasma GLP-1 concentrations [188]. The number of participants in this study was small (n = 10) and more evidence is required about the potential applicability of metformin in the management of PPH in patients with and without diabetes.

1.5.2.6 Other medications

Levodopa, combined with a peripheral decarboxylase inhibitor (PDI) such as carbidopa, is the most effective and most frequently used medication in the treatment of elderly patients with Parkinson's disease [189, 190]. Levodopa therapy has also been shown to slow gastric emptying, supporting the concept that dopaminergic therapy induces clinically important delays in gastric emptying [191, 192]. However, surprisingly, there have been no studies investigating the effect of an acute levodopa intake on gastric emptying in Parkinson's disease.

If levodopa is found to delay gastric emptying, this may represent a new therapeutic approach to the management of PPH, particularly in Parkinson's patients who are not on dopaminergic medication.

Numerous other pharmacologic agents have been explored in the treatment of PPH, but none of them has consistently shown benefit. These include midodrine (an α-1 agonist) [193, 194], dihydoergotamine mesylate [193, 194], denopamine (beta-1 agonist) [194], nitrendipine [12], hydrochlorothiazide [195], indomethacine, cimetidine, diphenhydramine [196], 5-hydroxytryptamine [197] and vasopressin [85].

1.6 Conclusions

This chapter represents a summary of current knowledge in relation to PPH, with a particular emphasis on the prevalence, clinical relevance, pathophysiology and management strategies. In this thesis, studies were performed to evaluate the following:

1. Longitudinal changes in the BP responses to, and gastric emptying of, an oral glucose load in healthy older subjects (**Chapter 4**).
2. Systematic review: Acute effects of nutritive and non-nutritive sweeteners on postprandial BP (**Chapter 5**).
3. Effects of ID administration of the artificial sweetener, sucralose, on BP and SMA blood flow in healthy older subjects (**Chapter 6**).
4. Acute effects of acarbose and gastric distension, alone and combined, on postprandial blood pressure in healthy older adults (**Chapter 7**).

5. Effects of guar and whey containing preload (Omniblend) on gastric emptying, glycaemia, small intestinal absorption and BP responses to oral glucose in healthy older subjects (**Chapter 8**).

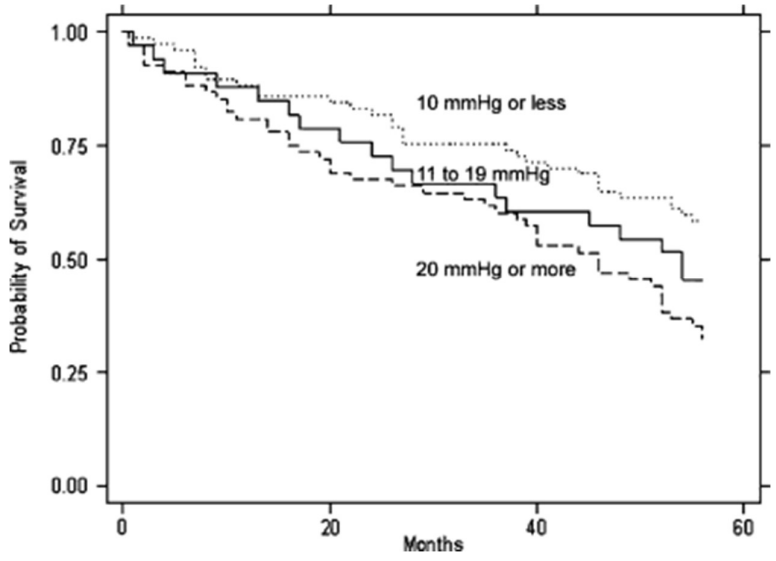

Figure 1.1. Kaplan-Meier survival analysis by degree of postprandial fall in systolic blood pressure. Log-rank test P = 0.009 [68].

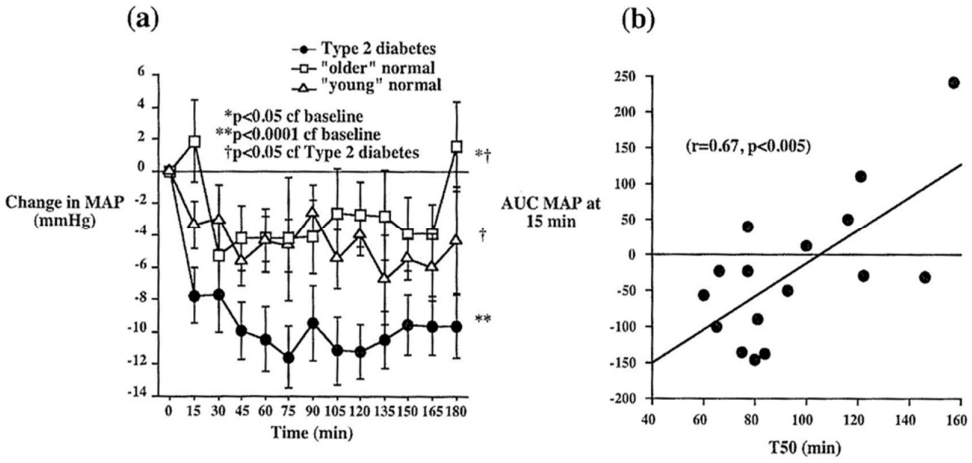

Figure 1.2. a) Change in mean arterial pressure (MAP) from baseline after a 75g glucose load in patients with T2D, as well as healthy young and older subjects and b) relationship between the area under the curve for the change in MAP between 0 – 15 min and the 50% emptying time of glucose in patients with T2D (n = 16). Data are mean values ± SE [7].

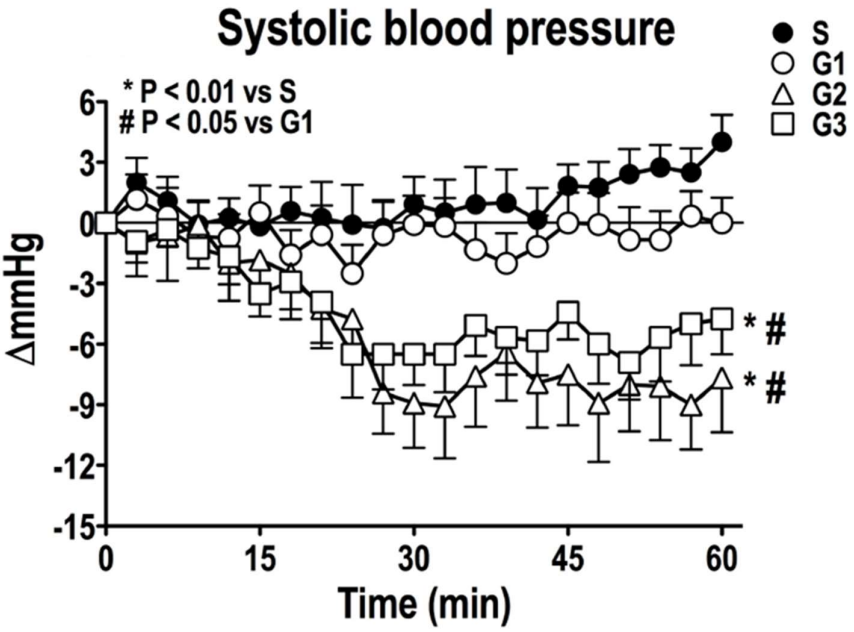

Figure 1.3. Effects of ID glucose at loads of 1 kcal/min (G1), 2 kcal/min (G2) 3 kcal/min (G3), or saline (S) on change in systolic BP in healthy older subjects (n = 12). Data are mean values ± SE [24].

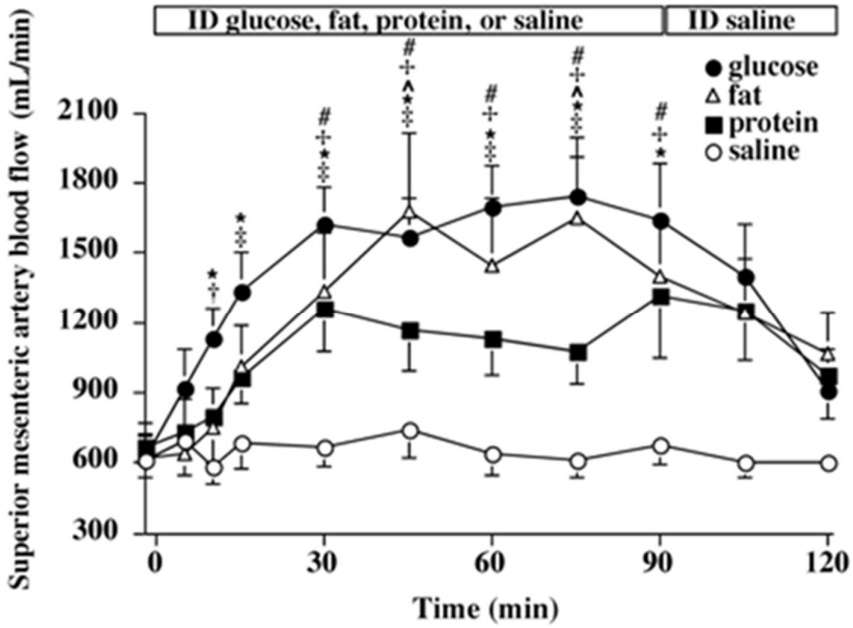

Figure 1.4. Effects of ID infusion of glucose, fat, protein and saline on SMA flow in healthy older subjects (n = 8) : *P < 0.01 for glucose compared with saline; †P = 0.04 for glucose compared with fat; ‡P < 0.05 for glucose compared with protein; P < 0.01 for fat compared with saline; #P < 0.05 for protein compared with saline; P < 0.01 for fat compared with protein. Data are mean values ± SE [9].

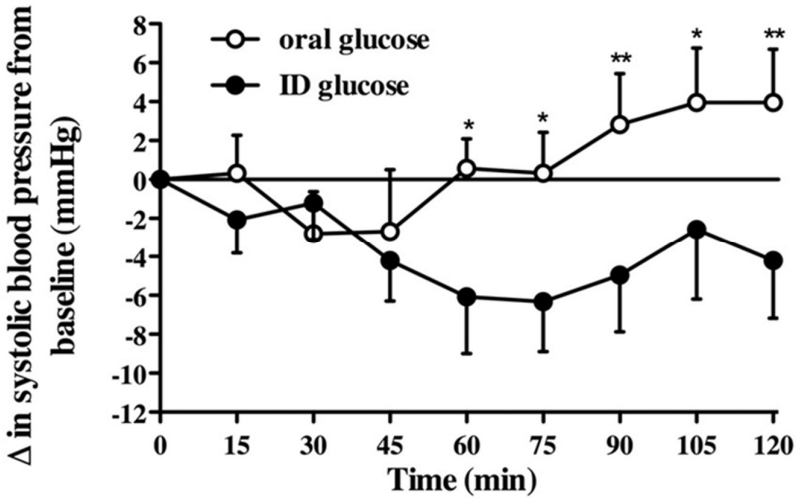

Figure 1.5. Changes (Δ) in systolic BP in 8 healthy older subjects after 75g glucose oral (○) and ID (•) glucose. Data are mean values ± SE [139].

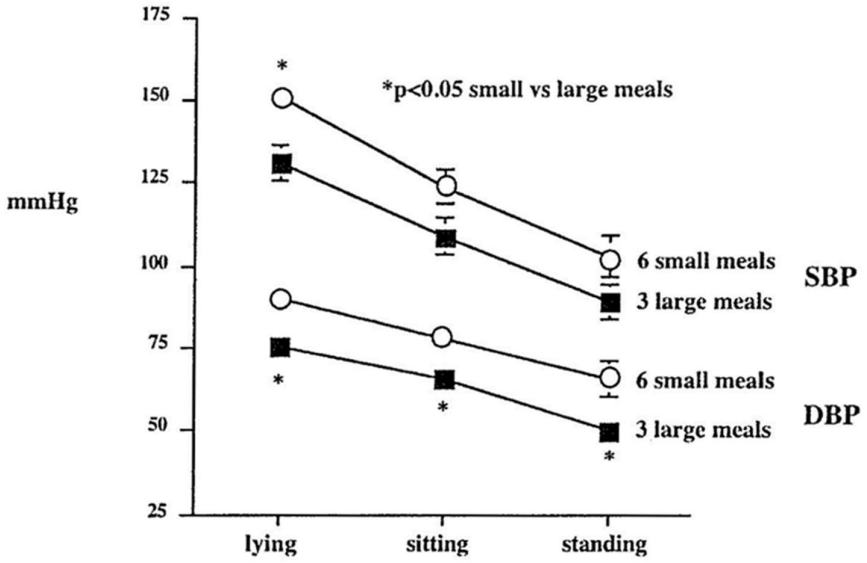

Figure 1.6. The effect of meal size on postprandial systolic (SBP) and diastolic (DBP) BP in patients with autonomic failure (n = 7). Data are mean values ± SE [13].

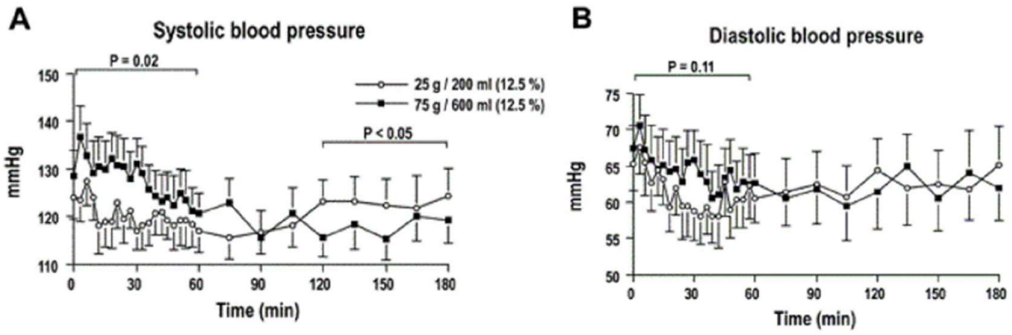

Figure 1.7. Effects of drink volume on systolic (A) and diastolic (B) BP after ingestion of 12% glucose drinks (25 g/200 mL vs. 75 g/600 mL) in healthy older subjects (n = 10). Data are mean values ± SE [140].

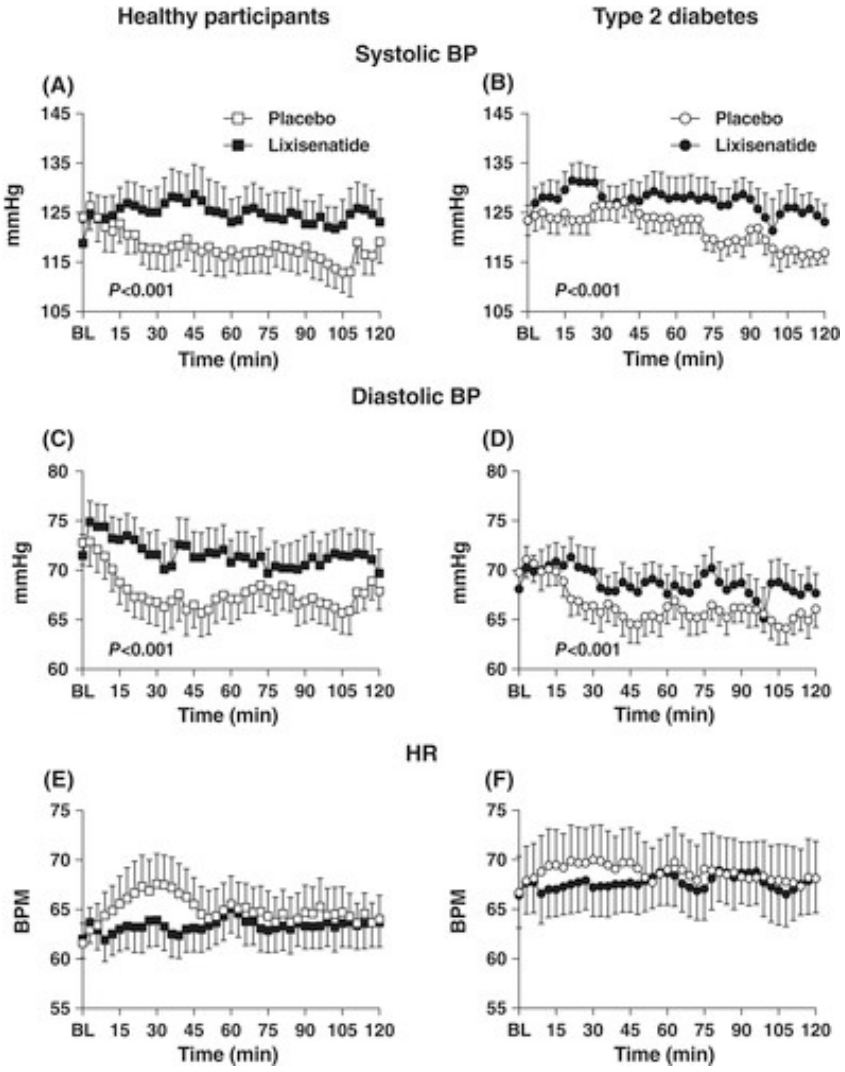

Figure 1.8. Effects of lixisenatide on (A, B) systolic blood pressure (BP), (C, D) diastolic BP and (E, F) heart rate (HR) responses to a 75g glucose drink in healthy participants (n = 15) and participants with T2D (n = 15). Data are mean values ± SE [111].

Chapter 2

Physiology of gastric emptying

2.1 Introduction

The primary functions of the stomach are to accommodate, mix and grind ingested food, and deliver nutrients into the small intestine at a regulated rate for their optimal digestion and absorption. This chapter reviews recent insights into gastric motor function, with a focus on the physiological role of the proximal and distal stomach, particularly in relation to gastric emptying.

2.2 Physiology of gastric emptying

Historically, substantial technical challenges compromised the extent of experimental investigation and, accordingly, the capacity to understand the physiology and pathophysiology of gastric emptying in humans [69, 198]. However, during the past 4 decades the development and application of sophisticated techniques, particularly, the 'gold standard' measurement of gastric emptying, scintigraphy, have provided fundamental information about normal and disordered gastric physiology [199]. Recently, several studies have provided novel insights into factors of importance to the physiology of gastric emptying, including neural loops, gastrointestinal hormones, smooth muscle and the interstitial cells of Cajal [200].

2.2.1 Role of the stomach

During the 'active' state when food is ingested into the stomach, gastric emptying can be divided into three separate, but inter-related, phases [69, 198, 200]:

(i) initial 'storage' of ingested food with increases in gastric compliance and fundic volume to accommodate food,

(ii) 'mixing' ingested food with gastric acid and pepsin and 'grinding' of solids to particles 1 - 2mm in size within the gastric antrum,

(iii) controlled ‘delivery’ of chyme into the small intestine, via coordinated antral and pyloric contractions regulated by inhibitory feedback arising from the small intestine.

The stomach can be divided into the proximal and distal segments by a mid-gastric transverse band, which may be functionally important in the intragastric redistribution of ingested solids [201]. In contrast to liquids which propagate quickly throughout the stomach, solids initially remain in the fundus, then are dispersed slowly towards the antrum by fundal rhythmic contractions [202, 203]. The integration of motor activity in the proximal stomach, antrum, pylorus and proximal small intestine [69, 204] generates bidirectional flow (antegrade and retrograde), which is variable between individuals [205] (Figure 2.1). This process is characterised by predominantly pulsatile, rather than continuous, movement of liquefied food (chyme) from the stomach into the duodenum [69, 203, 204]. Smooth muscles in different regions of the stomach vary in their contraction phenotype [200]. For example, while smooth muscles in the proximal stomach primarily exhibit tonic contractions, contractions are characteristically periodic in the distal stomach, and both tonic and periodic phenotypes are evident at the pylorus [200].

2.2.2 Proximal stomach

Food delivered from the oesophagus is initially retained in the fundus and upper part of the corpus of the stomach. During ingestion, vagally mediated transient ‘receptive’ relaxation is followed by the activation of the proximal stomach to accommodate ingested food. Therefore, an increasing volume of ingested food only causes minor elevations in intragastric pressure [202, 206, 207].

2.2.3 Distal stomach

In the fasted state, the antrum exhibits three patterns of motor activity with a duration of ~ 100 minutes: (i) quiescence (phase 1 with a duration of ~ 40 minutes), (ii) irregular contractions (phase 2, with a duration of ~ 50 minutes), and (iii) regular contractions at a frequency of ~ 3/min for 5-10 minutes, the so-called 'migrating motor complex' (MMC) [208] (Figure 2.2). Larger nondigestible solids > 2 mm in size are emptied during phase 3 of the MMC.

After solids have moved into the distal stomach, they are mixed and broken down into 1-2 mm particles by peristaltic contractions and gastric digestive fluids, before passing through the pylorus - so-called 'trituration' [208]. This process is modulated by a 'pacemaker' located high on the greater curvature at the boundary between the fundus and antrum, which discharges electrical signals at a rate of about three per minute [202, 203]. The 'pacemaker' represents a network of the interstitial cells of Cajal, found in the circular and longitudinal muscle layers within the gastric wall. Although the precise roles of the cells of Cajal are incompletely defined [209], these cells are likely to modulate gastric motility in a number of ways, including initiating and pacing slow waves [210], driving electrical signals into smooth muscle cells which induce slow waves [211] and serving as mechanosensors [212]. Distal stomach contractile activity is always associated with gastric slow waves [208], however, the slow-wave persists even in the absence of gastric contractile activity [208, 213]. Contractile activity is only initiated when excitatory neurotransmitters are released. In humans, the velocity of slow waves increases from proximal to the distal stomach - the velocity at the mid-corpus approximates 0.5 cm/second, increasing to 4 cm/second in the terminal antrum [214].

2.2.4 Pylorus and proximal duodenum

The pylorus serves as a brake to prevent particles larger than 2 mm from entering the duodenum [215] by exerting both tonic and phasic contractile activity over a narrow zone of approximately 2mm. Moreover, the pyloric sphincter and the proximal duodenum interact closely to stimulate or inhibit emptying during the digestive state [200, 216] - so-called "antroduodenal coordination" [200]. The pyloric sphincter closes to prevent further gastric emptying when the proximal duodenum contracts to pump the chyme aborally into the second segment of the duodenum. In contrast, the duodenum 'relaxes' to facilitate gastric emptying during antral contractions [200].

2.2.5 Patterns of gastric emptying

Normally, it takes approximately 2-3 hours for the stomach to empty the majority of both liquids and digestible solids during the digestive state. The small percentage of remaining unground food empties into the duodenum during the inter-digestive state [217]. The patterns of gastric emptying are strongly determined by the physical and chemical composition of ingested food and there is a striking difference between the emptying of liquids and solids from the stomach [69, 202].

2.2.5.1 Solids

Following a meal, solids are confined within the proximal stomach which functions as 'the housekeeper' of the stomach. Solids are then emptied from the stomach through a biphasic pattern including the lag phase and the linear emptying phase. The lag phase lasts ~ 20 - 40 minutes during which solids are distributed from the proximal to the distal stomach and triturated into smaller particles (1–2 mm). The following phase occurs over 3–4 hours, almost

in a linear pattern, with digestible solids passing through the pylorus (Figure 2.3) [73, 202, 218]. The interaction between nutrients and duodenal receptors induces negative feedback in order to maintain a constant outflow from the stomach [202].

2.2.5.2 Liquids

Liquids are rapidly distributed within the stomach and their emptying commences essentially immediately following ingestion, with a minor, if any, lag phase. Emptying of non-nutrient liquids normally follows a mono-exponential pattern (Figure 2.3) reflecting the effects of intragastric volume and gravity [218]. In contrast, emptying of densely nutrient liquids follows a linear pattern similar to that of solids, but without a lag phase. The stomach is able to process gastric emptying of solids and liquids separately [219]. Liquids empty preferably so that ~ 80% empties before solid emptying commences so that emptying of solids is delayed by liquids.

2.3 Regulation of gastric emptying

The regulation of gastric emptying is complex and a number of interactive mechanisms generate inhibitory small intestinal feedback. As a result of this, nutrients are normally delivered into the duodenum at a rate between 1– 4 kcal/min [220, 221].

2.3.1 Neural regulation

Although the stomach possesses its own intrinsic neural plexuses that allow it to operate autonomically to a certain degree via the MMC, extrinsic neural signals arising from the central nervous system through parasympathetic and sympathetic pathways are also important [222]. While the sympathetic nervous system is primarily responsible for inhibiting intraluminal muscle activity and mucosal secretion and modulating blood flow, the parasympathetic nervous

system exerts both inhibitory and excitatory effects to regulate gastric emptying (ie. tone, contractility) through the inhibitory and excitatory vagal loops [200, 222, 223]. The inhibitory vagal motor loop is fundamental to the regulation of gastric motility, while the excitatory loop contributes to the release of gastric acid and hormones [222]. Inhibition of the MMC in the stomach by food induces vagal stimulation [200, 222, 224].

2.3.2 Nutrient composition

The rate of gastric emptying when expressed as kcal emptied per min, in a given individual, is relatively predictable irrespective of the volume and caloric content of a meal - but the interindividual variation is much larger. The exposure of nutrients to luminal receptors distributed throughout the small intestine creates neurohormonal feedback. Various nutrients (i.e. glucose, fatty acids, and amino acids) and their characteristics (i.e. acidity, osmolarity) are sensed by specific small intestinal receptors with differences in regional distribution, number and type [208, 225]. Using magnetic resonance imaging to quantify gastric emptying of three macronutrients, Goetze et al. reported that while glucose and protein were emptied comparably, the gastric emptying rate of fat was significantly slower [226]. This probably reflects the physical characteristics of fat which, as an ‘oil’ phase, has the capability to ‘layer’ on top of more dense meal components as determined by posture/gravity [227]. Intraduodenal infusion of triglyceride, protein or fat, induces a substantial slowing in the rate of gastric emptying by suppressing antroduodenal pressure waves, triggering local pyloric tonic and phasic contractions and attenuating the tone of the proximal stomach [228]. The magnitude of small intestinal feedback is determined by the length and region of small intestine exposed to nutrients [221]. Repeated nutrient exposure may also impact gastric emptying function through adaptive mechanisms [229]. For example, previous exposure to a high-fat diet is associated

with a faster gastric emptying rate of fat at subsequent meals [229]. This is also the case when the gastric emptying of glucose accelerates after short-term consumption of glucose [230, 231].

2.3.3 Hormonal regulation

The ingestion of macronutrients induces the release of several hormones from the gastrointestinal tract and other tissues [232-234]. These hormones can be divided into two groups according to their effect on the rate of gastric emptying: inhibitory hormones include cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1) and leptin, while ghrelin and motilin have stimulatory properties [200]. CCK release from the intestinal I cells largely results from digestion of dietary lipid into long-chain fatty acids [235], and inhibits the secretion of both gastrin and gastric acid while also decreasing gastrointestinal motility by activating vagal afferent fibres [235, 236]. GLP-1 is released from L cells in the small intestine and colon; and as discussed in **Chapter 3**, stimulates insulin secretion while inhibiting glucagon secretion in a glucose-dependent fashion [119]. GLP-1 has been shown to increase fasting and postprandial gastric volumes while slowing gastric emptying [237], possibly reflecting stimulatory effects on vagal nitrergic pathways [237, 238] and vagal afferents originating from the gastrointestinal tract [238, 239]. Leptin is secreted from gastric chief (parietal) cells in the gastric mucosa [240] and has complex effects [241], interacting with the vagus nerve and CCK to delay gastric emptying in response to protein meals [241]. Leptin insufficiency is associated with an increased rate of gastric emptying [242]. Interestingly, the secretion of stimulatory hormones is temporarily inhibited during the inter-digestive state [200]; ghrelin and motilin are only secreted when there are no nutrients remaining in the stomach or duodenum to expedite gastric emptying [200, 243]. Ghrelin, released from G cells of the stomach, stimulates gastric emptying of liquids and solids through effects on autonomic functions [243]. Agonists of ghrelin have been developed and may be of therapeutic benefit for the management of gastroparesis [243,

244]. Motilin is secreted from M cells of the stomach and accelerates gastric emptying through its interaction with gastric enteric neurons [243]. Agonists of motilin - the first characterised was the antibiotic, erythromycin [245] are in use, and development for the management of gastroparesis [246, 247].

2.3.4 Ageing and gastric emptying

Ageing is associated with a modest slowing of gastric emptying, however the rate still generally varies within the physiological range from 1-4 kcal/min [248-250]. Normal ageing is associated with impaired regulation of gastric emptying [249] - although the frequency of antegrade flow pulses decreases with age [251], the overall gastric contractile activity is well-preserved [250, 252]. Notably, there is a significant reduction in sensory response to mechanical distention, particularly at proximal stomach [250], due to age-related loss in enteric neurons and interstitial cells of Cajal [249, 250]. This neuron cell loss also results in decreased acid secretion (~30%) [253], and increased pre and postprandial CCK and GLP-1 levels [254, 255] which may be related to delayed gastric emptying, decreased ghrelin [256, 257] and increased leptin levels [249, 258].

2.4 Measurement of gastric emptying

There are several available techniques to quantify gastric emptying which can be divided into imaging and non-imaging groups. This section describes the most widely used techniques with a focus on the two techniques used in my research studies described in **Chapter 4, 8 and 9**: scintigraphy and the stable isotope breath test.

2.4.1 Scintigraphy

Scintigraphy, regarded as the 'gold standard' technique for the assessment of gastric emptying [259] and intragastric meal distribution [260]. The rate of gastric emptying can be determined by imaging radiolabelled meal components with a gamma camera. The duration of the study can range from as short as 60 minutes up to 4 hours, depending on the composition of the meal, to improve the diagnosis of disordered emptying [259]. Radioisotopic images are acquired anteriorly or posteriorly with a single-headed camera or simultaneous anterior and posterior images with dual-headed camera in the standing, sitting or lying position in either dynamic mode ie 30-60 sec frames for the first 30-60 min (to capture the so-called lag phase – the time immediately preceding that in which food empties into the small intestine), followed by 2-3 min frames for the remainder of the study following ingestion of a radiolabelled test meal [259]. Data are then corrected for subject movement, gamma-ray attenuation and radionuclide decay, depending on the radiopharmaceutical employed, and in the case of a dual-isotope study in which two different meal components eg solids and liquids, can be measured simultaneously using two different radioisotopes, data can be corrected for downscatter from one energy window into another. Regions of interest are drawn around the stomach manually or using an automated program and the percentage retention of the meal can be plotted to generate a gastric emptying curve where time zero is 100%. Despite its long-term and common use in clinical and experimental settings, the technique is associated with some limitations i.e. variations in test meal, duration of image acquisition and patient positioning, lack of availability in a rural setting and the technique incurs a radiation exposure [261].

2.4.2 Stable isotope breath test

The stable isotope breath test is a non-invasive, non-imaging method, representing an alternative to scintigraphy to measure gastric emptying [260]. This method utilises stable isotopes eg ^{13}C-octanoic acid, ^{13}C-spirulina platensis or ^{13}C-acetate to label solid or liquid meals. The method is indirect i.e. it relies on gastric emptying and subsequent absorption from the small intestine [260]. ^{13}C is subsequently oxidised in liver and expelled through lungs as CO2 [262]. Exhaled end-tidal breath samples are collected into sealed bags right prior to the ingestion of test meal and every 5 min for the first hour, and then every 15 min for up to the subsequent 3 hours. The time points and duration of breath sample collection can be adapted to different study protocols. Concentrations of ^{13}CO2 are measured using computer isotope ratio mass spectrometry [263]. A 50% emptying time (T50) can be calculated using the formula described by Ghoos et al. [264]. Compared to scintigraphy, the stable isotope breath has some advantages. It can be performed at the bedside or in a doctors office and there are no risks induced by radiation exposure, hence it can be used safely in pregnant and breastfeeding females and children. The test is much more economical and may be repeated several times with reasonable reproducibility [265]. However, some factors should be taken into account during the result interpretation. Given that it is an indirect technique, dependent on the involvement of multiple organs including the small intestine (absorption), liver (oxidation), and lungs (emission) [263]. While this technique has been shown to correlate with scintigraphy, it produces a longer gastric emptying time [266]. The accuracy of the test may also be compromised by conditions that alter the normal absorption rate of the small intestine [260, 263]. Therefore, the results of stable isotope breath tests should be considered notional rather than accurate [7].

2.4.3 Wireless motility capsule

The wireless motility capsule (WMC) is a non-digestible capsule orally ingested to monitor chronologically the change in pH, pressure and temperature of the gastrointestinal tract [263]. Gastric emptying is calculated indirectly based on the recorded increase in intragastric acidity [263]. Despite the advantage of a non-radiation method, this technique only demonstrates a modest agreement with data measured by scintigraphy [267, 268]. Furthermore, WMC data only reflects the phase III contraction of migrating motor complex (MMC), which is altered by hyperglycaemia and proven weaker in T2D, rather than gastric emptying because the device empties from the stomach with indigestible solids during the inter-digestive period [262]. Accordingly, further evaluation is warranted before the conclusion that it may be used alternatively in the assessment of gastric emptying can be drawn [268].

2.4.4 Ultrasound

Ultrasound providing real-time measurements of gastric distension and gastric emptying at fixed time intervals after a standardised solid/liquid meal has been used widely in research and clinical settings [269]. It has also been used to evaluate non-lumen occlusive antral contractility, and transpyloric flow [267]. 2D ultrasound involves measuring changes in the antral area to calculate gastric emptying, whereas 3D ultrasound can be used to assess changes in the volume of the entire stomach. The latter provides more accurate information on gastric emptying but requires an expensive 3D transducer and processing software [261]. Advantages of ultrasound include the absence of radiation exposure, lower running costs and quantitative information on intragastric distribution and volume [260, 261]. Ultrasound has been shown to be well correlated with scintigraphy in measuring the gastric emptying of liquids [270].

However, the use of this modality requires an experienced operator and can be associated with some technical difficulties e.g. the presence of bowel gas or when scanning obese subjects [261].

2.4.5 Magnetic resonance imaging

Magnetic resonance imaging (MRI) has the potential to become an imaging modality of choice in the assessment of both gastric emptying and gastric motility at the same time [271]. Compared to scintigraphy, it is not associated with a radiation burden and has a shorter acquisition time [271]. A previous study demonstrated a good correlation between MRI and scintigraphy in quantifying gastric emptying on lung transplant patients [271]. However, this technique remains primarily used in the research setting and further studies are required before it can be used widely for clinical purposes [261].

2.4.6 Acetaminophen absorption technique

Acetaminophen absorption, a non-imaging old-fashioned technique to measure gastric emptying, is based on the principles that no or very little, if any, acetaminophen is absorbed from the stomach and that there is a linear correlation between the acetaminophen absorption rate into the bloodstream and gastric emptying [272]. This technique does not require any special equipment. However, the test result primarily presents the gastric emptying rate of liquids [261] and a portion of paracetamol is emptied from the stomach much quicker than the meal [273]. Furthermore, this technique is affected by variations in several extragastric factors such as first-pass hepatic metabolism, volume of distribution, and elimination of acetaminophen [261]. Accordingly, it should not be considered an accurate method.

2.5 Conclusions

This chapter outlines the current understanding of normal gastric motor function and gastric emptying. The major roles of the stomach are to initially store and then convert ingested food into chyme, as well as regulate gastric emptying to deliver nutrients into the duodenum. Gastric emptying is a rhythmically pulsatile process and regulated tightly and primarily by small intestinal feedback. Gastric emptying is now considered a major determinant of the postprandial fall in blood pressure as discussed in **Chapter 1**, and postprandial glycaemia as discussed in **Chapter 3**, while the effects of ageing on gastric emptying in relation to postprandial hypotension (PPH) and incretin hormones are explored in **Chapter 4** and **Chapter 9** respectively.

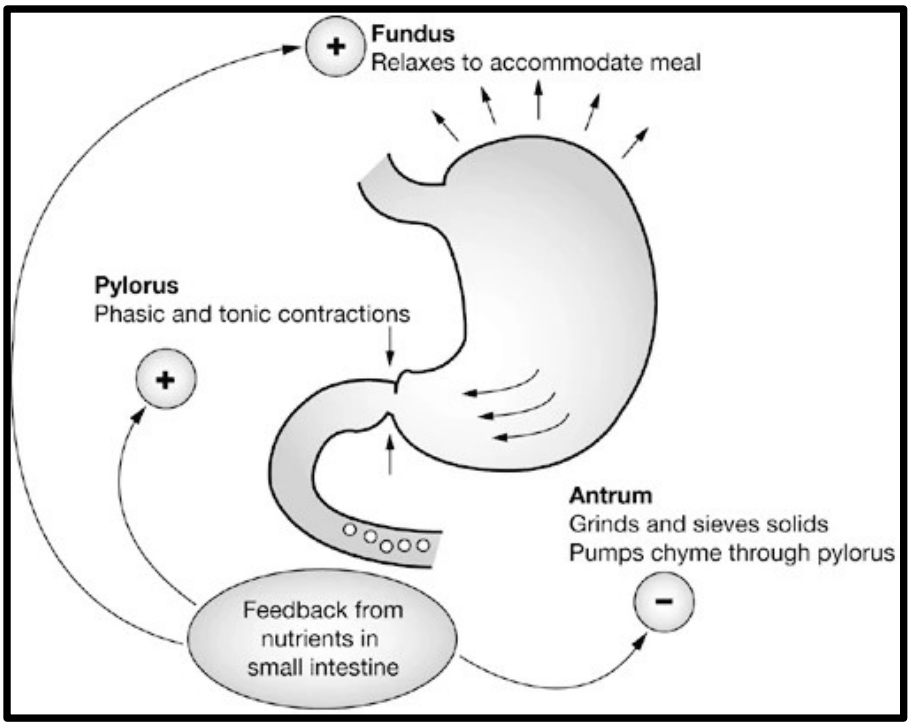

Figure 2.1. Motor events during normal gastric emptying. The fundus relaxes to accommodate the meal, while the antrum grinds and sieves solids, pumping the resultant chyme into the duodenum against resistance generated by phasic and tonic pyloric contractions. The presence of nutrients in the small intestine generates neurohumoral feedback on gastric motor function, enhancing fundic relaxation and pyloric contraction, while suppressing antral motility, with the net effect that further emptying is closely regulated [198].

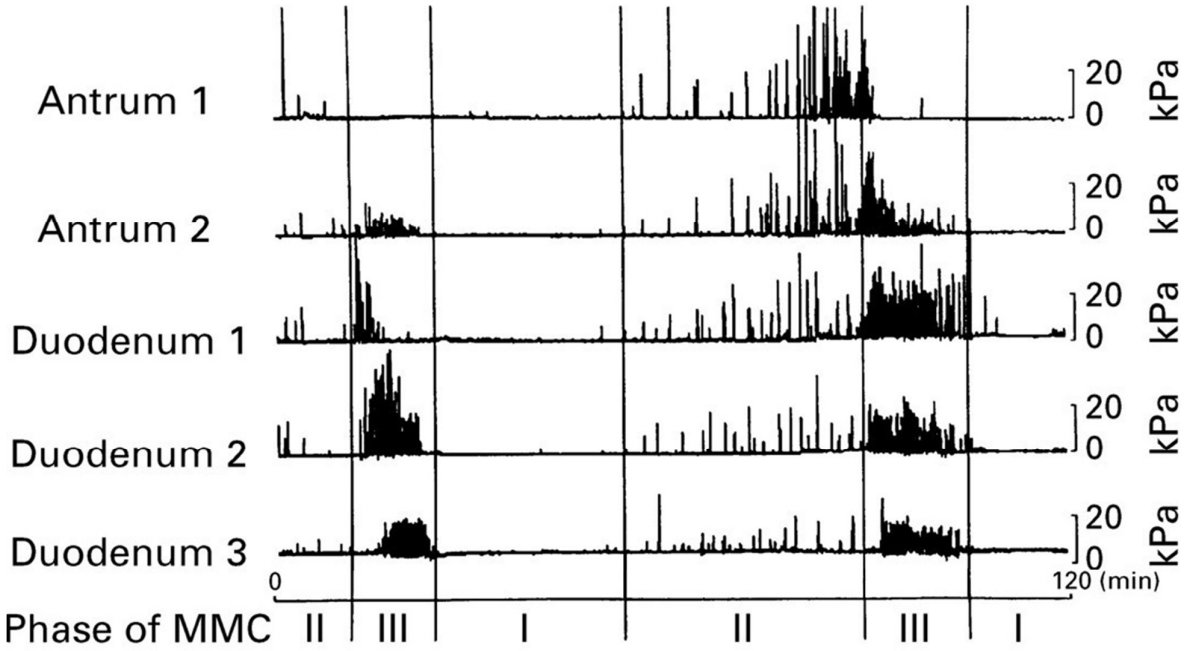

Figure 2.2. Schema of migrating motor complex (MMC) which occurs periodically (1–2 hour cycle) during the digestive state and is characterised by three phases usually arising in the antrum: contractile activity is absent during phase I, irregular activity occurs during phase II, and there are intense, regular coordinated contractions during phase III [274].

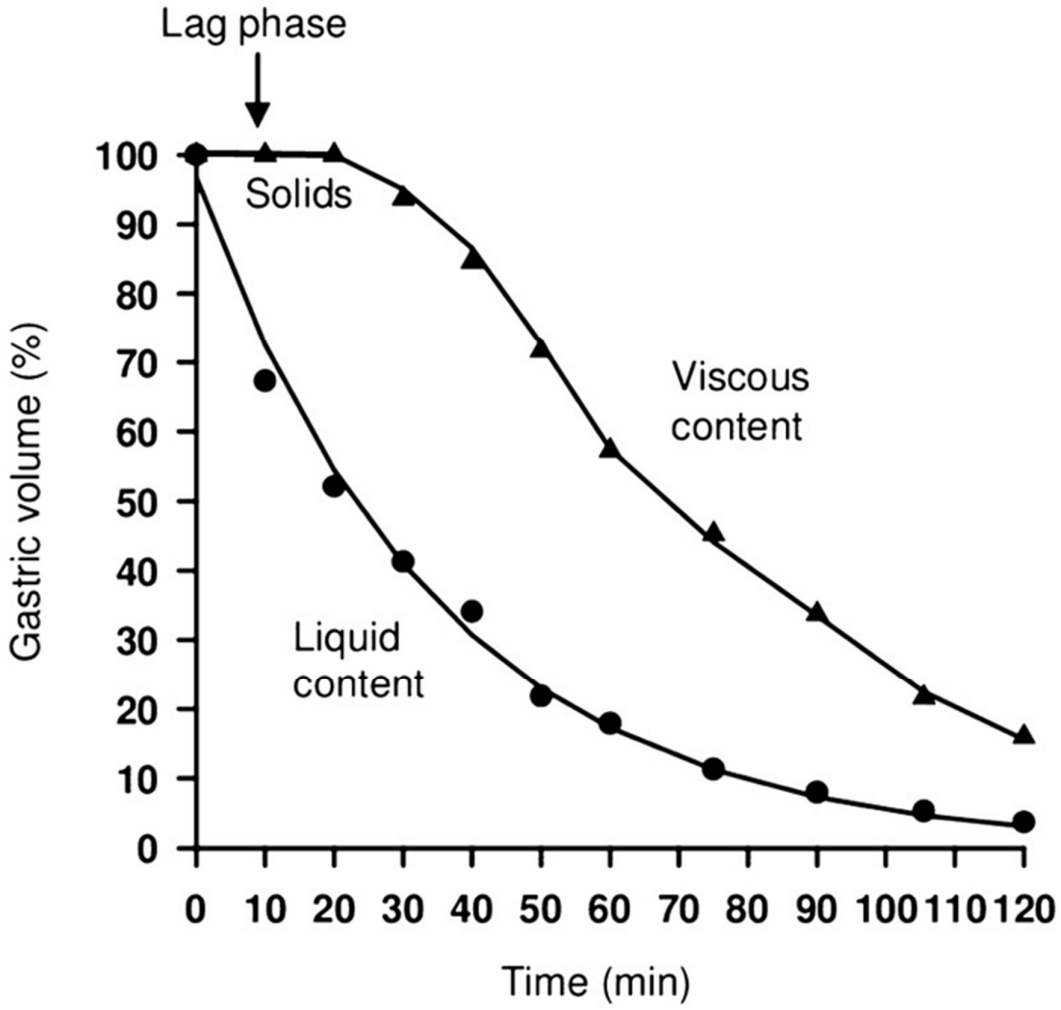

Figure 2.3. Gastric emptying curves for a solid (▲) and liquid (●) meal in a healthy volunteer. Liquid emptying begins immediately and follows an exponential fashion, while the solid emptying is linear after an initial lag phase [275].

Chapter 3

Gastric emptying and glycaemia

3.1 Introduction

Type 2 diabetes (T2D) is characterised by dysfunctional nutrient metabolism that leads to an abnormally increased glucose concentration. While the pancreatic hormones, insulin and glucagon, play major roles in regulating both fasting and postprandial glycaemia, it is now appreciated that the regulation of blood glucose homeostasis is complex, involving multifactorial interactions between the brain-gut axis, liver, kidney, gastrointestinal tract, and muscle. This chapter focuses on the interdependent relationship of gastric emptying with glycaemia in healthy and diabetes that are relevant to the studies pursued by the PhD candidate and reported in **Chapter 9**.

The gastrointestinal tract is recognised as crucial to the regulation of plasma glucose concentrations, which are tightly controlled in health even though there is a substantial difference in glucose concentrations between the inter-digestive (fasting) and intra-digestive (fed) states. In the inter-digestive state, the plasma glucose concentration represents the balance between the release of endogenous glucose and glucose uptake by body organs as determined by insulin and glucagon secretion [276] and accounts for ~50% of the variance in blood glucose concentrations [276]. Food intake drives more complex glucose-regulatory processes given that absorbed nutrients represent an exogenous glucose source [277]. Insulin secretion from pancreatic beta cells increases sharply to transport glucose into peripheral tissues and suppresses glucose generation by the liver. In addition, glucagon released from pancreatic α cells is suppressed to also limit the magnitude of the postprandial rise in blood glucose [277], which is determined by the surplus of glucose production and glucose disposal. In healthy subjects, blood glucose concentrations rise approximately 10 minutes after ingestion of a carbohydrate-containing meal to usually achieve a maximal value within the first hour [278]. If the maximal rise in blood glucose occurs after 2 h, it is suggestive of disordered glucose

disposal into peripheral issues [278]. The gastrointestinal tract accounts for two pivotal contributions to postprandial glucose regulation - the 'incretin effect' and the rate of gastric emptying (Figure 3.1).

3.2 The incretin effect - GLP-1 and GIP

The 'incretin effect' refers to the phenomenon by which oral, or enteral, administration of glucose results in a substantially greater insulin secretory response when compared to an intravenous infusion resulting in the same glucose concentrations [279], accounting for 50 – 70% of total insulin secretion following oral glucose in healthy subjects [119] (Figure 3.2). The interaction between nutrients and the small intestine signals enteroendocrine cells to release the two known incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). K cells, located predominantly in the proximal small intestine, secrete GIP primarily in response to glucose or fat ingestion [119]; while L cells, located predominantly in the distal ileum and colon, secrete GLP-1 in response to fat, carbohydrate, protein and bile acids [280, 281].

The insulinotropic effects of both hormones are glucose-dependent, requiring a 'threshold' blood glucose > 8 mmol/L [119]. In addition, GLP-1 suppresses, while GIP may stimulate, glucagon secretion. While GIP has little or no effect on gastric emptying [282], GLP-1 delays gastric emptying [282] and enhances satiety to reduce food intake [119]. The circulating half-lives of GLP-1 and GIP are very short (2 min and 5-7 min respectively) and both are degraded quickly by the ubiquitous enzyme, dipeptidyl peptidase-4 (DPP-4) [119]. In healthy subjects and patients with T2D [283-285], exogenous GLP-1 slows gastric emptying of liquids and solids in a dose-dependent fashion, by suppressing antro-duodenal contractility, enhancing the pyloric tone and relaxing the proximal stomach [286]. Accordingly, GLP-1 reduces, rather than

increases, postprandial insulin concentrations [285]. The magnitude of the slowing of gastric emptying by exogenous administration of GLP-1 diminishes with sustained administration for ∼ 24 h, i.e. there is tachyphylaxis, but the magnitude of the slowing is still substantial at this time [287].

In T2D the insulinotropic action of exogenous GLP-1 is relatively maintained [288]. In contrast, the insulinotropic effect of GIP, which may be the dominant incretin in health, is markedly attenuated [119, 289, 290]. This has stimulated the development of GLP-1 based medications for the management of T2D. Currently, two GLP-1 therapies are in use [291]: oral DPP-4 inhibitors (e.g. sitagliptin, vildagliptin and alogliptin) [292], which enhance the effects of endogenous GLP-1 by inhibiting its breakdown, and subcutaneous GLP-1 receptor agonists (e.g. exenatide, liraglutide and lixisenatide) [293], which mimic the effects of endogenous GLP-1 by binding to GLP-1 receptors but are resistant to DPP-4 induced biodegradation [291]. Based on their half-lives, GLP-1 receptor agonists can be categorised as 'short-acting' (e.g. exenatide twice daily and lixisenatide) and 'long-acting' (e.g. exenatide once weekly and liraglutide) [291, 294]. 'Short-acting' GLP-1 receptor agonists have a dominant effect to reduce postprandial glycaemia, while with 'long-acting' agonists effects on preprandial glucose predominate [295]. With GLP-1 agonists, the risk of hypoglycaemia is low because of the glucose-dependency of insulinotropic and glucagonostatic effects [296]. DPP-4 inhibitors have broader effects than GLP-1 receptor agonists because of influences on other gastrointestinal hormones including glucose-dependent insulinotropic peptide (GIP), glucagon-like peptide-2 (GLP-2) and peptide YY (PYY).

3.3 Impact of gastric emptying on glycaemia and incretin hormones

Small intestinal nutrient delivery, which in health is tightly regulated at a rate of approximately 1-4 kcal per minute [297], is an important determinant of the early (~15 – 60 min) and overall glycaemic responses to a meal so that gastric emptying accounts for up to ~35% of the fluctuation in the glycaemic response to oral glucose in health [298, 299], type 1 diabetes (T1D) and T2D [300, 301]. In T1D and T2D, gastric emptying is more variable - abnormally delayed gastric emptying occurs frequently in longstanding, poorly-controlled diabetes [302, 303], whereas, as a group well-controlled T2D is associated with relatively more rapid emptying [304] (Figure 3.3). The relationship between late (~120 – 180 min after a glucose load) glycaemia with gastric emptying is dependent on the timing of insulin secretion triggered by the early increase in blood glucose, e.g. in healthy subjects the relationship between the 120 min glucose and gastric emptying after a 75g glucose load is inverse, rather than direct [299, 301].

Several studies employing intraduodenal administration of glucose at rates within a physiological range of 1–4 kcal/min have established that the correlation between glycaemia with small intestinal delivery is non-linear in healthy subjects [305, 306] and patients with diet-controlled T2D [307]. The increase in blood glucose is moderate after an intraduodenal infusion of glucose at 1 kcal/min. In contrast, 2 kcal/min, 3 kcal/min, and 4 kcal/min infusions induce much more prominent glycaemic responses with relatively little difference between them (Figure 3.4). These findings can be attributed to a substantially greater insulin response to the higher glucose infusions (i.e. ≥ 2 kcal/min) which reflects the secretion of GLP-1 and GIP [308] - GLP-1 secretion is only substantial at intraduodenal glucose loads > 2 kcal/min [306] whereas that of GIP is linear. Accordingly, it is likely that when gastric emptying is ≤ 2 kcal/min, GIP accounts for a greater contribution to the incretin effect, which is outweighed by

GLP-1 at higher rates of gastric emptying ≥ 3 kcal/min [306], Consistent with this, Marathe et al. demonstrated that the magnitude in the incretin effect is dependent on the intraduodenal glucose load in healthy subjects, as well as patients with T2D [308]. However, with the same overall intraduodenal load, the stimulation of the incretin effect and insulin secretion by a greater early small intestinal glucose delivery only induces a greater insulin concentration at the early stage compared to a constant infusion, but does not affect the total glycaemic response [309].

Gastric emptying has been modulated by dietary and pharmacological means to improve glycaemic control in T2D - these strategies including nutrient 'preloads', 'short-acting' GLP-1 receptor agonists and the amylin analogue, pramlintide, which all slow gastric emptying [149, 310-312]. This approach has been stimulated by the recognition that the major determinant of glycated haemoglobin, a marker of the risk of microvascular complications of diabetes in patients with 'reasonable' glycaemic control (i.e. 7 < HbA1c ≤ 8%), is postprandial glycaemic excursions and that the latter need to be normalised to achieve good control.

3.4 Effects of glycaemia on gastric emptying

The relationship between gastric emptying and glycaemic response is bidirectional, i.e. postprandial glucose concentrations also impact on gastric emptying [297, 313]. Acute hyperglycaemia - even variations in glucose within the normal postprandial range [314] - slows gastric emptying, while acute insulin-induced hypoglycaemia accelerates it substantially [315] (Figure 3.5). The blood glucose concentration should, accordingly, be quantified during clinical measurements of gastric emptying in people who have diabetes [261] and it is recognised that hyperglycaemia may attenuate the response to drugs which accelerate gastric emptying [316] and potentiate the response to drugs, including GLP-1, which may slow it [317]. High plasma

glucose concentrations are known to be associated with the presence of gastric electrical dysrhythmias [318] and inhibition of both pre and postprandial antral motor activity [318-320] although the underlying mechanisms remain uncertain [321]. The acceleration of gastric emptying by hyperglycaemia is likely to represent an important counter-regulatory response by increasing the rate of carbohydrate [315, 322].

3.5 Measurement of blood glucose concentrations

Venous blood sampling is the easiest and most frequently used method to quantify blood glucose concentrations [323]. In the fasting state, glucose concentrations measured from arterial, capillary and venous blood are highly comparable, whereas the difference between arterial and venous blood concentration is up to 20% in postprandial state [324]. This arteriovenous discrepancy has been shown to be higher in healthy people compared to diabetic patients. Capillary blood glucose concentrations have been shown to correlate well with arterial blood glucose ones [325]. However, the use of capillary blood is hindered by a requirement of multiple finger pricks for multiple consecutive measurements. The arterialised-venous technique, achieved by warming the arm used for venous blood sampling, is applied to utilise the convenience of venous sampling method as well as to obtain more comparable readings with capillary blood [323].

3.6 Conclusion

The relationship of gastric emptying with postprandial glycaemia is important and interrelated, and modulated by several factors, particularly the incretin hormones. Gastric emptying is a major determinant of postprandial glycaemic excursions which represent the dominant contribution to overall glycaemic control (i.e. HbA1c < 8.0%) in diabetes which has stimulated

the development and widespread application of strategies to improve glycaemic control in T2D by slowing gastric emptying.

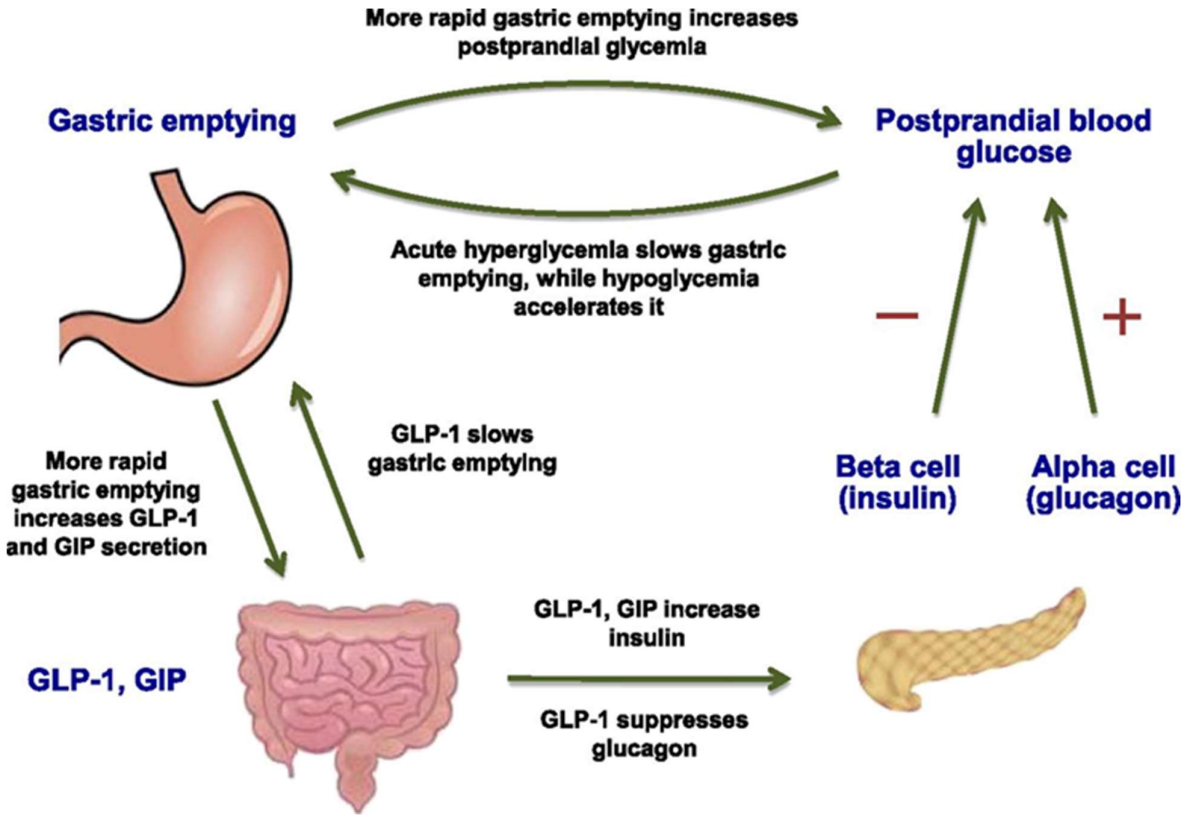

Figure 3.1. Summary of the interdependent relationships of gastric emptying, incretin hormones, and postprandial glycaemia [326].

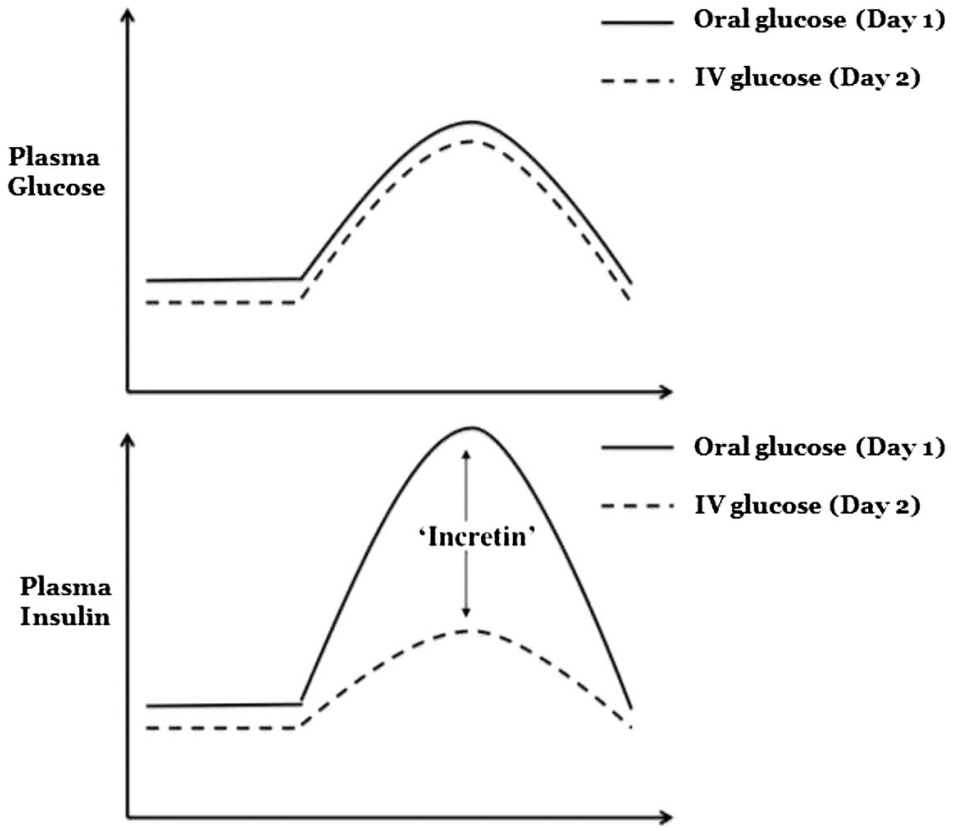

Figure 3.2. The incretin effect. There is a much greater release of insulin in response to oral glucose administration as compared with administration of an isoglycaemic intravenous (IV) glucose infusion [327].

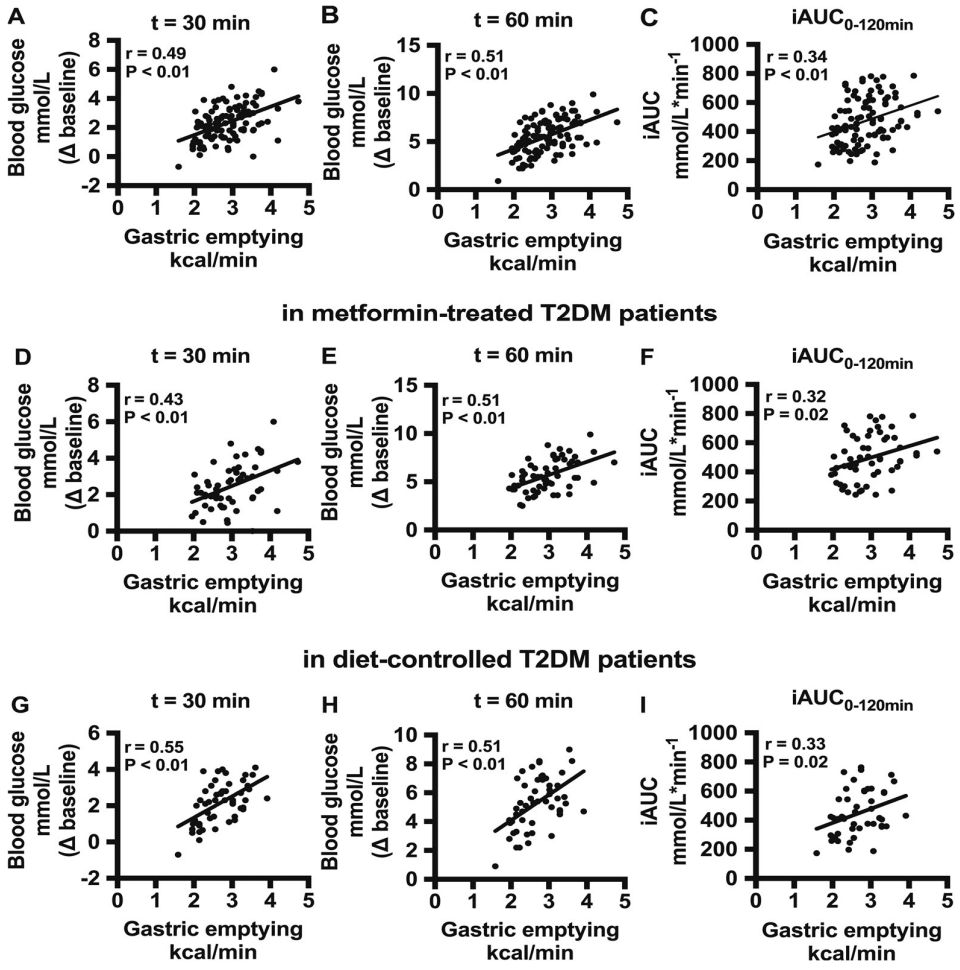

Figure 3.3. (A–C) Relationship between the blood glucose increment (at t = 30 and 60 min after the meal and the iAUC for blood glucose during 0 to 120 min) and the rate of gastric emptying in all patients with T2D. (D–F) Relationship between the blood glucose increment (at t = 30 and 60 min after the meal and the iAUC for blood glucose during 0 to 120 min) and gastric emptying in the patients with T2D taking metformin. (G–I) Relationship between the blood glucose increment (at t = 30 and 60 min after the meal and the iAUC for blood glucose for 0 to 120 min) and gastric emptying in the patients with T2D without metformin [304].

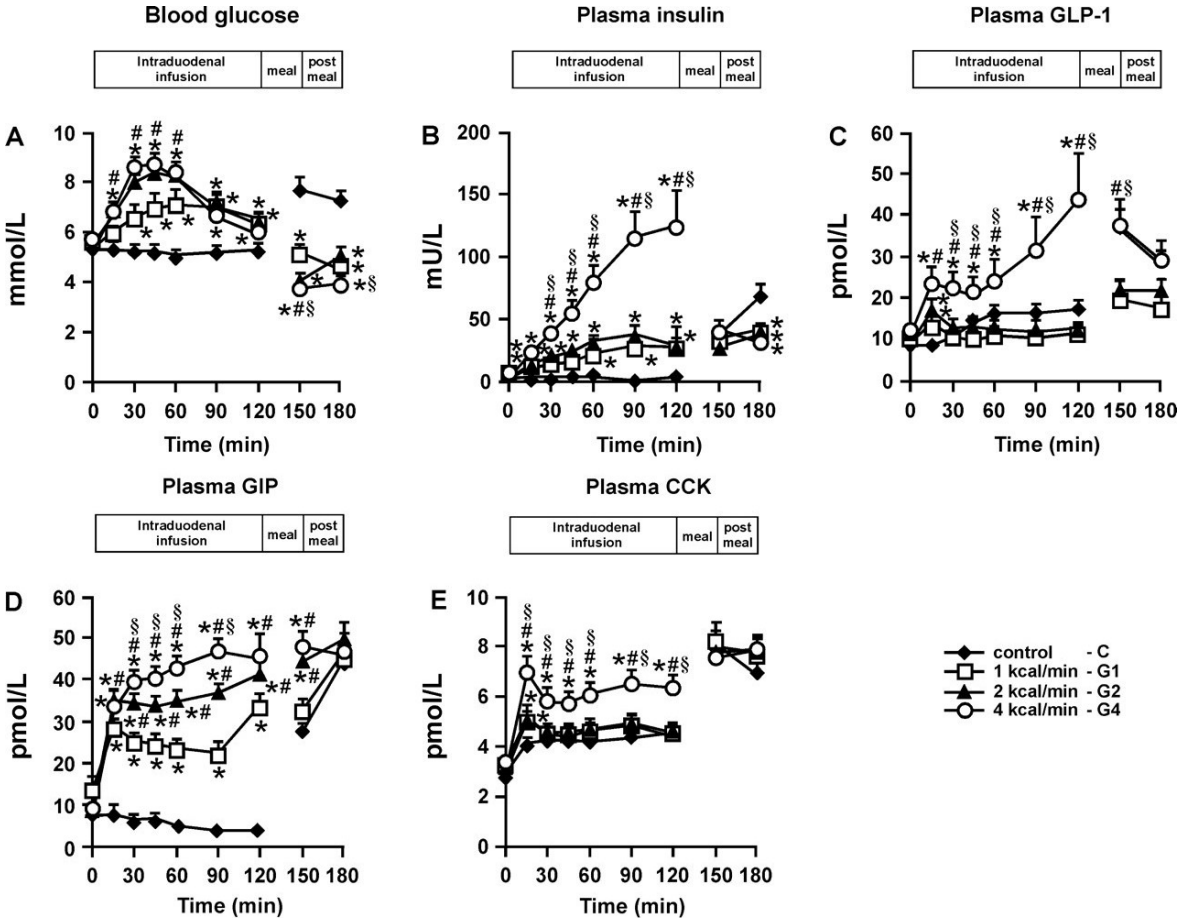

Figure 3.4. Blood glucose (A) and plasma insulin (B), glucagon-like peptide-1 (GLP-1; C), glucose-dependent insulinotropic polypeptide (GIP; D), and cholecystokinin (CCK; E) concentrations in response to 120-min intraduodenal glucose (25%, 1,390 mosmol/l) infusions at 1 ("G1"), 2 ("G2"), or 4 ("G4") kcal/min or saline (4.2%, 1,390 mosmol/l) control ("C") in 10 healthy males. Data are means ± SE. A: *P < 0.05 vs. control. #P < 0.05 vs. G1. §P < 0.05 vs. G2. B: *P < 0.05 vs. control. #P < 0.05 vs. G1. §P < 0.05 vs. G2. C: *P < 0.05 vs. control. #P < 0.05 vs. G1. §P < 0.05 vs. G2. D: *P < 0.01 vs. control. #P < 0.05 vs. G1. E: *P < 0.05 vs. control. #P < 0.01 vs. G1. §P < 0.01 vs. G2 [305].

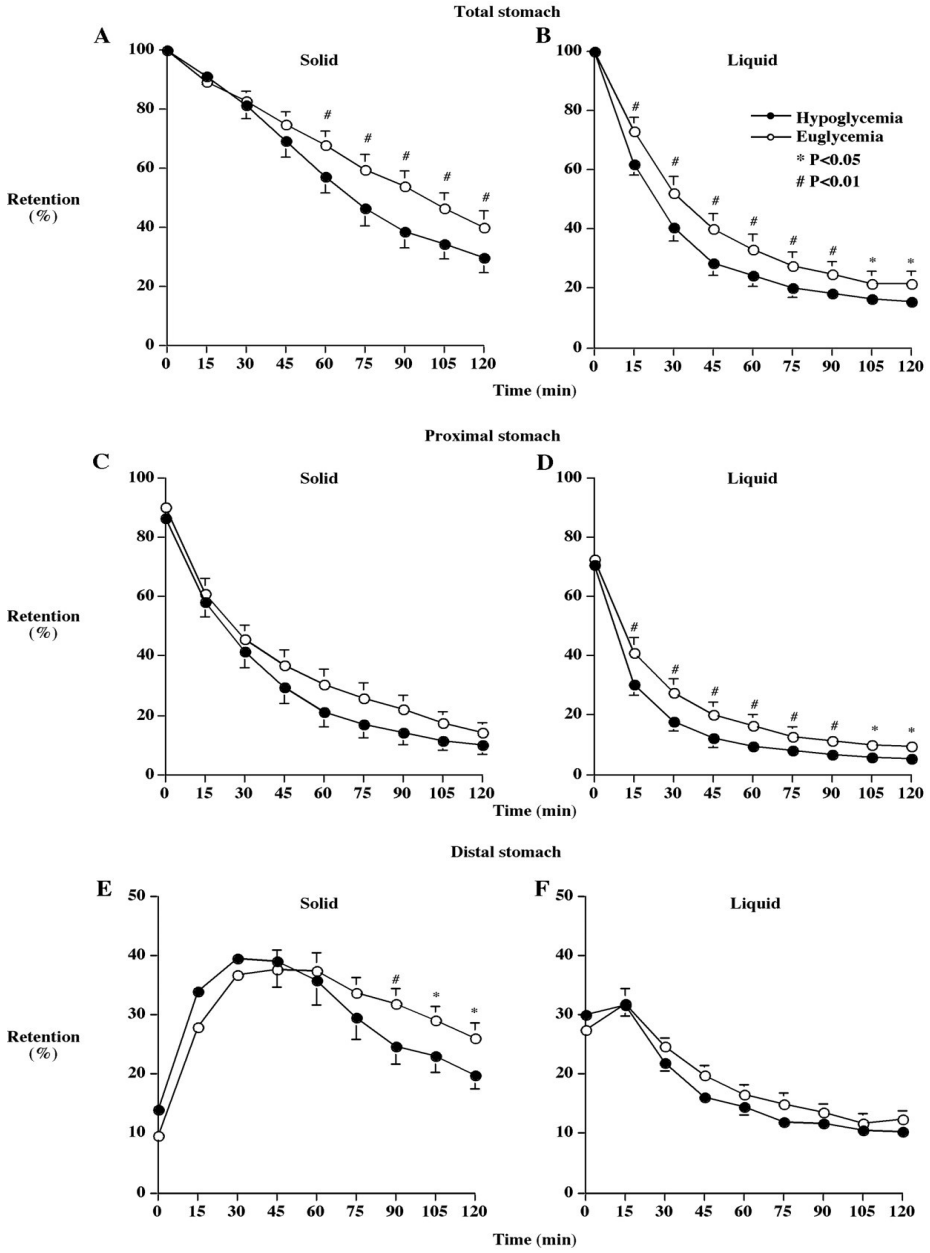

Figure 3.5. Gastric emptying and intragastric distribution of the solid (left) and liquid (right) components of the meal (100 g lean minced beef labelled with 20 MBq ^{99m}Tc-sulfur colloid chicken liver and 150 mL water labelled with 7 MBq ^{67}Ga-EDTA) during hypoglycaemia (black circles) and euglycaemia (white circles) in patients with T1D. Data are mean ± SEM. *, $P < 0.05$; #, $P < 0.01$ compared with euglycaemia (ANOVA) [315].

Chapter 4

Longitudinal changes in the blood pressure responses to, and gastric emptying of, an oral glucose load in healthy older subjects

Statement of Authorship

Title of paper	Longitudinal changes in the blood pressure responses to and gastric emptying of, an oral glucose load in healthy older subjects.
Publication Status	Published
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Principle Author

Name of Principal Author (Candidate)	Hung T Pham		
Contribution to the Paper	Conducted research, analysed and interpreted data, wrote and revised the paper.		
Overall percentage	70%		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	Aug 2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Liza K Phillips		
Contribution to the Paper	Conceived and designed research, interpreted data and reviewed paper.		
Signature		Date	Aug 2019

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4.1 Introduction

Postprandial hypotension (PPH), diagnosed by measurement of the blood pressure (BP) response to oral glucose or a meal [1], is now recognised as an important clinical problem, particularly in healthy people (prevalence 13-38%) [5, 74] and patients with autonomic dysfunction, often secondary to type 2 diabetes (T2D) (~35%) [1, 5] or Parkinson's disease (40-100%) [1, 5]. PPH is associated with a number of adverse sequelae, including syncope and falls [1, 4] and is an independent risk factor for death [68].

While the pathophysiology of PPH is complex and incompletely understood, it is clear that the interaction between autonomic mechanisms, the release of gastrointestinal hormones, gastric distension, and small intestinal nutrient delivery are all important in the regulation of postprandial BP. After a meal, there is an approximate doubling of superior mesenteric artery (SMA) blood flow [1], which, in health, is accompanied by concomitant increases in heart rate (HR), peripheral vascular resistance, stroke volume and cardiac output [5]. In patients with PPH, these compensatory responses are inadequate to maintain BP [1]. The rate of gastric emptying, which exhibits a substantial inter-individual variation [220], is a major determinant of the hypotensive response to a meal [7, 8, 24]. We have shown that in T2D patients [7] and healthy older people [74], the postprandial fall in BP is greater when gastric emptying is more rapid. Although cross-sectional studies suggest that healthy ageing is associated with a modest slowing of gastric emptying [328], longitudinal studies have hitherto not been reported. While ageing is known to be associated with an increase in BP in older people [329, 330], there is no information about the hypotensive response to oral nutrients, or the natural history of PPH. A cohort of healthy older participants was re-evaluated after an interval of ~ 5.8 years to determine changes in the BP response to, and gastric emptying of, oral glucose, as well as the relationships between the hypotensive response to glucose with gastric emptying.

4.2 Materials and methods

4.2.1 Participants

Eighty-six older individuals who took part in an initial study (July 2010 - July 2012) evaluating BP, glycaemia and gastric emptying of a 75g oral glucose load [74] were invited by mail to participate in this follow-up study. Of the original cohort, 33 participated; 8 had medical conditions that precluded their involvement, 10 were taking antihypertensive drugs which represented an exclusion, 13 refused to participate, 21 did not respond to the letter of invitation and in 1 case, the invitation letter was returned and the individual considered to be lost to follow-up. Of the 33 individuals (17 female; 16 male) who agreed to return, the mean age at the initial study was 71.0 ± 0.7 years and body mass index (BMI) 25.6 ± 0.5kg/m ^{2}. At 'follow-up' (mean interval 5.8 ± 0.1 years) age was 77.0 ± 0.7 years and BMI 26.2 ± 0.5kg/m^{2}.

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Human Research Ethics Committee of the Royal Adelaide Hospital.

4.2.2 Protocol

The protocol was identical to that undertaken at the initial study [74]. Individuals presented at ~08.30h after an overnight fast (14h for solids; 12h for liquids) [74]. They were seated in an armchair, an intravenous cannula was inserted into an antecubital vein for blood sampling and an automated BP cuff placed around the opposite arm. After a 'rest period' of 15-30 min, individuals consumed a drink containing 75g glucose and 150mg ^{13}C-acetate (Cambridge Isotope laboratories, Tewksbury, MA, USA), made up to 300mL with water. Time zero (t = 0) was defined as the time of completion of the drink. Measurements of gastric emptying, BP and

HR were performed at regular intervals until t = 120 min [1]. At the end of the study, participants were offered a light lunch.

4.2.3 Measurements

4.2.3.1 Blood pressure and heart rate

Systolic BP (SBP), diastolic BP (DBP) and HR were measured with an automated oscillometric BP monitor (DINAMAP ProCare 100, GE Medical Systems, Milwaukee, WI, USA) every 3 minutes during the 'rest period', and every 5 minutes until t = 120min. Baseline (fasting) BP was calculated as an average of the measurements obtained at t = -9, t = -6, and t = -3min. PPH was defined as a fall in SBP≥20 mmHg that was sustained for ≥30 min [7].

4.2.3.2 Gastric emptying

Exhaled breath samples were collected before ingestion of the drink (t = -3min), every 5 minutes for the first hour (commencing at t = 5 min) and then every 15 minutes for the subsequent 3 hours, for assessment of gastric emptying. The $^{13}CO_2$ concentration in the breath samples was measured by an isotope ratio mass spectrometer (ABCA 20/20; Europa Scientific, Crewe, UK), and the gastric 50% emptying time (T50) calculated [264].

4.2.4 Statistical Analysis

BP, HR and gastric emptying were analysed and presented as absolute values. The maximum falls in SBP and DBP, and rise in HR was defined as the greatest change from baseline between 0-120min and 60-120min. One-way ANOVA was used to analyse the effects of time on the change from baseline values for BP and HR. Areas under the curve AUCs) were calculated using the trapezoidal rule and differences between the initial study and follow-up were assessed

using Student's paired t-test. Pearson's correlation was used to evaluate relationships between variables. All analyses were performed using SPSS version 24 (SPSS, Chicago, IL, USA). A P-value < 0.05 was considered significant in all analyses. Data are presented as mean values ± SEM.

4.3 Results

The studies were well tolerated and there were no adverse events. In 7 subjects, a nonlinear regression model fit to the measured $^{13}CO_2$ concentrations was not feasible. Accordingly, paired BP and HR data were available in 33 participants, while gastric emptying data were available in 26 participants.

4.3.1 Blood pressure and heart rate

Three subjects (9.1%) at the initial study and six subjects (18.2%) at follow-up had PPH. Two subjects had PPH on both studies. The third subject with PPH at the initial study exhibited a maximum fall in SBP of more than 20mmHg at the follow-up, but this was not sustained for 30 min. There was an increase in baseline SBP from the initial study to follow-up (120 ± 2.5 vs 128 ± 2.2mmHg, P = 0.001), with no change in baseline DBP (68 ± 1.4 vs 69 ± 1.0mmHg, P = 0.58) or HR (64 ± 1.3 vs 63 ± 1.4bpm, P = 0.65).

4.3.1.1 Systolic blood pressure

Following the drink, there was a modest rise, followed by a fall (P < 0.001 for both) in SBP on both study days (Figure 4.1A). Between t = 0-120min, there was a difference (P = 0.001) in the AUC of SBP, such that SBP was greater at follow-up compared with the initial study. There was no significant difference in the maximum fall in postprandial SBP between the initial and

follow-up studies over the 2-hour follow-up (-13.6 ± 1.6 vs -15.8 ± 1.6mmHg, P = 0.18), however, the maximum fall in postprandial SBP between t = 60-120min was significantly greater at follow-up (-11.7 ± 1.4 vs -15.2 ± 1.6 mmHg, P = 0.04) (Figure 4.1A).

4.3.1.2 Diastolic blood pressure

Following the drink, there was a transient, modest rise, followed by a fall (P < 0.001 for both) in DBP on both study days (Figure 4.1B). There was no difference (P = 0.53) in the AUC of DBP between the initial study and follow-up, nor was there any difference in the maximum fall in postprandial DBP over the 2-hours (-11.8 ± 0.8 vs -11.8 ± 0.6mmHg, P = 0.99) nor the maximum fall in postprandial DBP between t = 60-120min (-10.8 ± 0.8 vs -10.9 ± 0.7 mmHg, P = 0.84) (Figure 4.1B).

4.3.1.3 Heart rate

Following the drink, there was no change in HR at the initial study (P = 0.53) or at follow-up (P = 0.45) (Figure 4.1C). There was no difference (P = 0.72) in the AUC of HR between the initial study and follow-up, nor was there any difference in the maximum rise in HR (8.1 ± 1.0 vs 8.8 ± 0.9bpm, P = 0.57) nor the maximum rise in postprandial HR between t = 60-120min 5.4 ± 1.0 vs 7.5 ± 1.0 bpm, P = 0.09) (Figure 4.1C).

4.3.2 Gastric emptying

Gastric emptying (T50) was slower at the follow-up study than at the initial study (137.9 ± 5.4 vs 153.8 ± 8.6min, P = 0.04) (Figure 4.2).

4.3.3 Relationships among blood pressure and gastric emptying

There were correlations between the AUCs of SBP ($R = 0.64$, $P < 0.001$) and between the maximum falls in SBP ($R = 0.48$, $P = 0.004$) (Figure 4.3A) between the two studies. At both the initial study and at follow-up, there was an inverse relationship between the maximum fall in SBP and baseline SBP, i.e. when baseline SBP was greater, the maximum fall was also greater ($R = -0.68$, $P < 0.001$ vs $R = -0.50$, $P = 0.003$). Moreover, the change in the magnitude of the maximum fall in SBP from the initial study to follow-up was related directly to the increase in baseline SBP between the two study days ($R = -0.63$, $P < 0.001$) (Figure 4.3B).

There was a relationship between gastric emptying at the initial study and at follow-up ($R = 0.53$, $P = 0.005$). There were also relationships between the AUC of SBP and gastric emptying at the initial study ($R = 0.54$, $P = 0.005$; Figure 4.3C) and at follow-up ($R = 0.41$, $P = 0.04$; Figure 4.3D) between t = 0-120min, i.e. the fall in SBP after the drink was less when the T50 was greater.

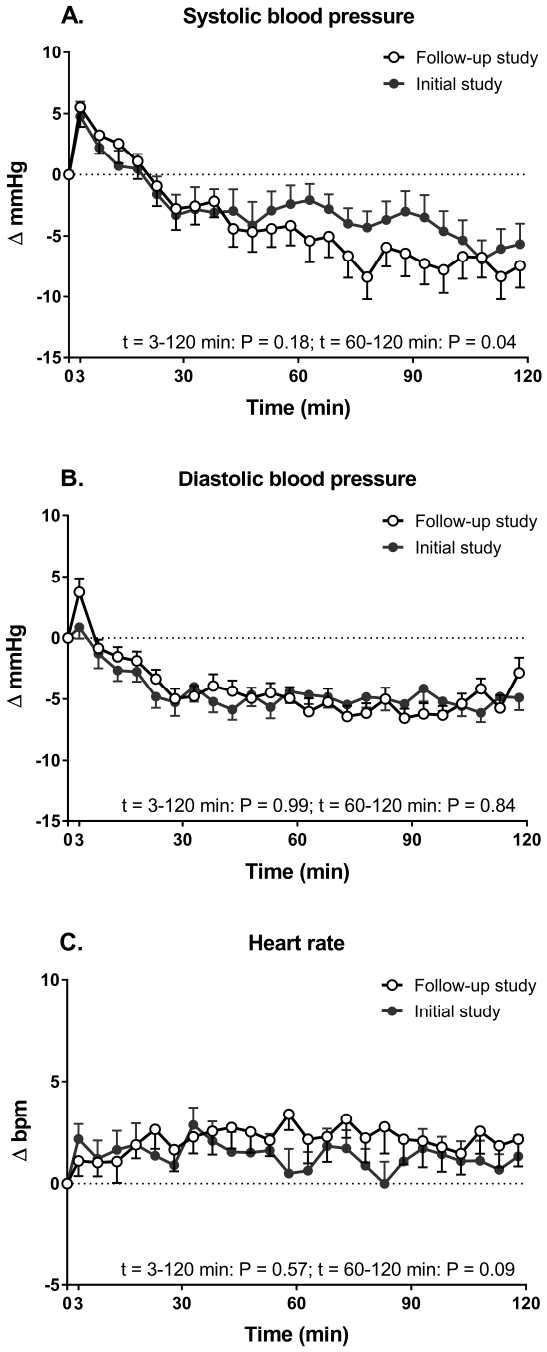

Figure 4.1. SBP (A), DBP (B) and HR (C) in older subjects following a drink containing 75g glucose at the initial study and follow-up (after ~5.8 years). Data are presented as mean change from baseline (fasting) values ± SEM (n = 33).

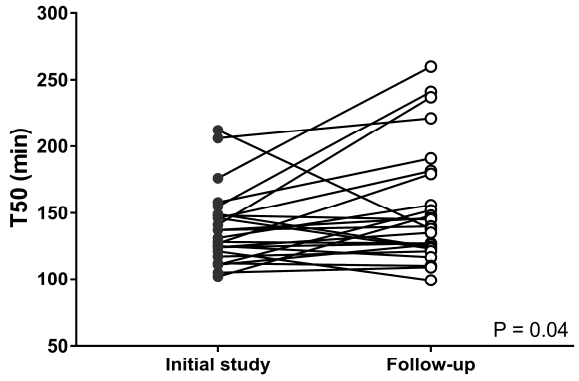

Figure 4.2. 50% gastric emptying time (T50) after 75g glucose at the initial study and follow-up (n = 26).

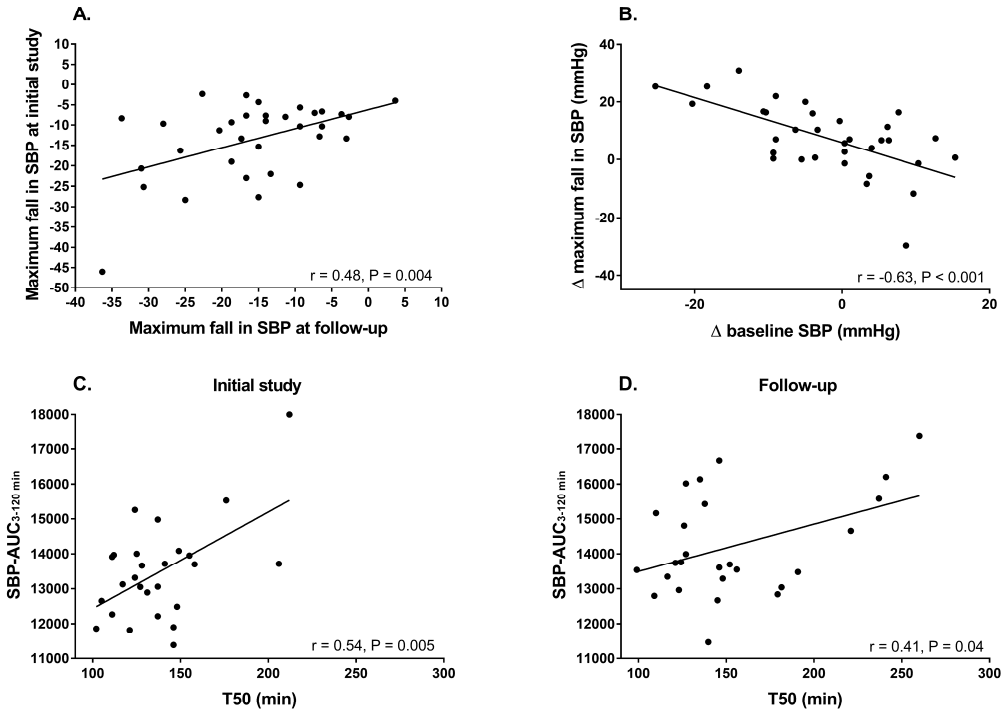

Figure 4.3. Relationship between (A) the maximum falls in SBP after a drink containing 75g glucose at the initial study and follow-up (n = 33), (B) the change in the magnitude of the maximum fall in SBP and the increase in baseline SBP from the initial study to follow-up (n = 33), (C) the $AUC_{0\text{-}120min}$ of SBP and the gastric emptying (T50) at the initial study and (D) the $AUC_{0\text{-}120min}$ of SBP and the gastric emptying (T50) at follow-up (B) (n = 26).

4.4 Discussion

This study represents the first longitudinal evaluation of the BP responses to, and gastric emptying of, an oral glucose load in healthy older people. Over a mean period of ~5.8 years, there was a modest increase in SBP and, consistent with the initial cross-sectional study, a significant relationship between the magnitude of the drink-induced fall in SBP with the rate of gastric emptying. Important novel observations are that the hypotensive effect of glucose was greater, and gastric emptying slower, with increased age. Moreover, the hypotensive response was greater when baseline (fasting) SBP was relatively higher. The prevalence of PPH also increased.

Many studies have reported that resting SBP rises with age, probably primarily reflecting a reduction in the wall elasticity of large arteries after age ~60 years. In contrast, DBP may decrease [329, 330]. Accordingly, the observed significant increase in baseline SBP at follow-up was predictable, although we failed to observe any change in DBP. As observed in our initial study, there was a sustained fall in SBP following the glucose drink and the magnitude of the fall in BP was greater when baseline SBP was greater [63, 331]. This is not surprising given that the frequency of PPH is known to be greater in hypertensive older people [1]. In a study in Europe including 530 older patients with isolated systolic hypertension, up to 70% exhibited a substantial fall in postprandial BP [332]. It is also not surprising that the magnitude of the hypotensive response in elderly people was greater after 5.8 years. Approximately twice as many subjects had PPH when compared to the initial study. Normal ageing is associated with a reduction in the sensitivity of the arterial baroreceptor reflex, which may account for the greater hypotensive response by leading to a reduction in cardiac output [333].

Hitherto, there has been little information about the natural history or intra-individual reproducibility of PPH. Puisieux et al. (2002) reported that 15 (30%) of 50 geriatric patients had PPH on two consecutive morning tests when PPH was defined as a SBP decline ≥20mmHg within 2h of a meal [334]. In our follow-up study, the intra-individual reproducibility of PPH over ~5.8 years was 66.7%, which was higher than the previous study [334] even though we used more stringent criteria to diagnose PPH which also considered the sustainability of the fall in SBP [7]. Jansen et al [335] reported a high level of reproducibility in measurements of the maximum falls in SBP in consecutive measurements within 2 weeks (0.88 (95% confidence intervals (CI) 0.85-0.97)), but this study was performed in nursing home residents, where the prevalence of PPH is known to be much higher than in community-dwelling healthy older adults.

Previous cross-sectional studies suggest that healthy ageing is associated with a modest slowing of gastric emptying [328], consistent with the findings in our longitudinal follow-up study. The mechanisms underlying the modest slowing are uncertain [328]. Similarly, the clinical relevance of this slowing, if any, remains to be determined. Gastric emptying is known to modulate the pharmacokinetics of many orally administered medications [336], as well as postprandial glycaemia [326] and appetite [328]. In relation to the latter, it is now recognised that healthy ageing is associated with physiological anorexia, which is likely to predispose to pathological weight loss [328]. This follow-up study confirms that although gastric emptying was slightly slower, the magnitude of the fall in SBP is related to the rate of gastric emptying. In other words, the faster gastric emptying, within the normal range, the greater the postprandial fall and the higher the risk of PPH. Hence, although our study establishes that changes in gastric emptying are not responsible for the increased prevalence of PPH with age, this has clear

implications for management providing a rationale for the use of dietary and/or pharmacologic strategies to slow gastric emptying [22-24].

While this study represents the first longitudinal evaluation of the BP response to, and gastric emptying of, oral glucose in healthy older people, the limitations should be appreciated. The size of the cohort was relatively small, baseline BP was normal in most cases and only 38.4% of those studied initially either agreed to participate or were excluded due to antihypertensive medication, introducing the potential for selection bias. Specifically, it would not be surprising if the participants who returned for follow-up represented a "super-healthy" cohort and, if so, the prevalence of PPH over a ~5.8-year period may be greater than estimated. Because the prevalence of PPH was only 18.2% the relationship of the hypotensive response to glucose with gastric emptying was evaluated in the group as a whole. Gastric emptying was measured by the indirect breath test technique where measurements should be regarded as notional, rather than precise (e.g. the observed gastric emptying T50 was longer than would be anticipated with scintigraphy [7]), but this has been shown to correlate closely to scintigraphy, the 'gold standard' technique [337, 338]. Gastric emptying of solids was not evaluated, but measurement of gastric emptying of solids and high-nutrient liquids appears to have comparable sensitivity. We did not assess patterns of nutrient intake, but there was no change in body weight.

This study establishes that in healthy older people the prevalence of PPH increases within ~5.8 years and that the rate of gastric emptying, which was slower, is a determinant of the hypotensive response.

Chapter 5

Acute effects of nutritive and non-nutritive sweeteners on postprandial blood pressure

Statement of Authorship

Title of paper	Acute effects of nutritive and non-nutritive sweeteners on postprandial blood pressure.
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Principle Author

Name of Principal Author (Candidate)	Hung T Pham		
Contribution to the Paper	Conducted a systematic search, reviewed literature, wrote and revised paper.		
Overall percentage	80%		
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	Aug 2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Liza K Phillips		
Contribution to the Paper	Conducted a systematic search, reviewed literature, wrote and revised paper.		
Signature		Date	Aug 2019

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Contribution to the Paper	Conceived review, reviewed paper and approved final version of paper.		
Signature		Date	Aug 2019

5.1 Introduction

The consumption of nutritive sweeteners has dramatically increased worldwide due to increasing urbanisation and beverage promotion [339]. Nutritive sweeteners commonly found in foods and beverages include glucose, fructose and sucrose [340, 341]. More recently, some other sweeteners like D-xylose, xylitol, erythritol, maltose, maltodextrin, stevia and tagatose with lower energy have been used [340, 341]. There has been a long history of debate as to the detrimental health effects of sucrose, glucose, and more recently, fructose, particularly when consumed in excess. The effects of both nutritive and non-nutritive sweeteners on cardiovascular risk factors, including blood pressure (BP), have been extensively investigated, however, less is known about their specific effects in the postprandial state. Postprandial hypotension (PPH), a fall in systolic BP (SBP) ≥ 20 mmHg after a meal, is now recognised as a frequent and important clinical problem, with a reported prevalence of 24-48% in the healthy elderly [5]. PPH is particularly common in conditions associated with autonomic dysfunction e.g. in type 2 diabetes mellitus (T2D) (~40%) [1, 5] and Parkinson's disease (PD) (40-100%) [1, 5]. Patients with PPH are at increased risk of syncope and falls [63], and importantly the presence of PPH has been established as an independent predictor of increased mortality [68]. In a cohort study of 401 elderly ambulatory hypertensive subjects, 292 (72.8%) were found to have PPH, and over a 4 year period, 34 died from cardiovascular disease; the post-breakfast BP fall was the strongest predictor of mortality in this cohort [67]. In a 29-month prospective study of 499 nursing home residents, the maximum fall in postprandial BP was shown to be an independent predictor of subsequent falls, syncope, new cardiovascular events (myocardial infarction and stroke) and total mortality [3].

The pathophysiology of PPH is multifactorial and incompletely understood, however, involves the interplay between autonomic and neural mechanisms, including the release of gut

hormones, which are influenced by meal composition, gastric distension, and small intestinal nutrient delivery [5]. After a meal, there is a doubling of the superior mesenteric artery (SMA) blood flow [1] and in healthy young individuals with intact baroreflex function, the increase in splanchnic blood flow is accompanied by concomitant increases in heart rate (HR), peripheral vascular resistance, stroke volume and cardiac output [12]. In patients with PPH, these compensatory responses are inadequate [1]. The postprandial fall in BP is greater when gastric emptying is more rapid [7, 70, 155], whereas gastric distension attenuates the fall in BP in both healthy young and older participants [7, 70, 155]. The macronutrient composition of the meal is also an important determinant of postprandial BP, with carbohydrate inducing an earlier fall in BP compared with protein and fat [9] (Figure 5.1). The interaction between nutrients and the small intestine stimulates the secretion of the incretin hormones, glucagon-like peptide-1 (GLP-1) from L-cells and glucose-dependent insulinotropic polypeptide (GIP) from K-cells [342]. GLP-1 stimulates insulin secretion, inhibits glucagon secretion and slows gastric emptying [109, 283]. Acute infusion of GLP-1 has been shown to attenuate the fall in BP and increases SMA blood flow following oral [109] or intraduodenal (ID) glucose [106, 109]. In contrast, exogenous GIP has been shown to increase the rate of gastric emptying [282], although results have been conflicting [343] and there have been no studies directly investigating the effect of GIP on postprandial BP. A reduction in BP is not observed following intravenous administration of glucose [102], which is a substantial stimulus to insulin secretion, suggesting that insulin by itself if not an important mediator of PPH. Furthermore, PPH is seen in the absence of insulin secretion i.e. in those with type 1 diabetes (T1D) [37].

In recent years, due to several negative health effects from the excessive consumption of added sugar, a variety of non-nutritive sweeteners have increasingly been used in the food industry as an alternative to sugar among people of all ages, particularly directed to the obese and those

with diabetes, with the aim of reducing energy intake and minimising the risk of obesity, diabetes and cardiovascular disease [341, 344]. Beverages account for the highest percentage of non-nutritive sweetener utilisation in the world [345, 346]. In the United States, non-nutritive sweeteners were used in up to 32% and 19% of beverages consumed by adults and children respectively from 2007 to 2010 [347]. In 2018, the American Heart Association suggested that short-term use of 'non-nutritive' sweeteners instead of 'nutritive sweeteners' can reduce caloric intake in the management of overweight and obesity [348].

There are some differences between regulatory bodies regarding approval of nutritive and non-nutritive sweeteners. The six non-nutritive sweeteners approved by Food and Drug Administration (FDA) as food additives in the United States include sucralose, Acesulfame-K, aspartame, saccharin, neotame and advantame [340, 349, 350]. Stevia [350, 351] has not received official FDA approval but has been rated as generally recognised as safe (GRAS) and is being increasingly used as a non-nutritive sweetener [352]. Maltitol similarly has not received official FDA approval, but may be used in food manufacture [352]. The nutritive sweeteners currently approved by the FDA include mannitol, xylitol, sorbitol and erythritol [353]. The European Food Safety Authority (EFSA) has approved the non-nutritive sweeteners Acesulfame-K, aspartame, cyclamate, neohesperidin dihydrochalcone, saccharin, sucralose, neotame, thaumatin and stevioside, while approved nutritive sweeteners include erythritol, isomalt, lactitol, maltitol, mannitol, sorbitol and xylitol [352].

There have been a number of short-term studies evaluating the physiological responses to nutritive and non-nutritive sweeteners that have included assessment of BP. With the increasingly widespread use of non-nutritive sweeteners and the documented severe clinical sequelae of PPH, it is clearly important to ascertain the extent to which these agents decrease

postprandial BP. To date, a published systematic summary of the impact of nutritive and non-nutritive sweeteners on postprandial BP has not been performed. For this systematic review, the aim was to establish current scientific evidence of the effects of some of the most commonly used FDA-regulated nutritive and non-nutritive sweeteners on postprandial BP, as well as their potential applications in the management of PPH.

5.2. Approach

Based on the pathophysiology of PPH, a systematic review of the PubMed database was conducted up to March 2019 to identify articles related to the effects of different types of sweeteners on postprandial BP. The following keywords were used: “sweeteners” or “nutritive sweeteners” or “caloric sweeteners” or “sucrose” or “fructose” or “glucose” or “xylose” or “xylitol” or “erythritol” or "maltose" or "maltodextrin" or "tagatose" or “non-nutritive sweeteners” or “non-caloric sweeteners” or “sucralose” or “Acesulfame-K” or “aspartame” or “saccharin” or “stevia” or "steviol glycoside" or "neotame" or "advantame") and (“postprandial blood pressure” or “postprandial hypotension” or “hypotensive response”).

Screening of studies was performed initially by assessment of the relevance of the abstract by 2 independent reviewers. The reference lists of relevant articles were also reviewed. Study inclusion was based on the guidelines for preferred reporting items for systematic reviews and meta-analyses (PRISMA) [354].

5.3 Results

A total of 150 papers were identified from the database search. There were no duplicate papers. Of the 150 papers, papers without full text (n = 2), irrelevant papers (n = 55), animal studies (n

= 12), reviews (n = 9), papers that were unavailable in the English language (n = 7), letters to the editor or comments (n = 1), and case reports (n = 2) were removed, leaving a total of 62 papers which were reviewed. Figure 5.2 summarises the selection process.

5.4 Nutritive sweeteners

5.4.1. Glucose

Glucose is the most abundant monosaccharide and is synthesised from water and carbon dioxide through photosynthesis and concentrated to create starch [355]. Foods containing relatively higher proportions of naturally occurring glucose include some fruits, such as grapes and bananas (~6-8%), while the glucose content of honey may approach ~38% [356, 357]. It is an essential energy source for the red blood cells and brain, although the latter can also utilise ketone bodies [340, 358]. Ingested complex carbohydrates are hydrolysed to monosaccharides before being absorbed [358]. The majority of studies investigating PPH have utilised an oral [7, 19, 22, 33, 36, 40, 42-45, 50-52, 54, 70, 74, 79, 104, 109, 111, 124, 126, 128, 133, 134, 139, 140, 144, 155, 158, 159, 161, 188, 359-365], and/or ID glucose [8, 9, 15, 23, 24, 75, 76, 83, 88, 90, 106, 180, 197, 366-369] load that induces a substantial fall in BP. There have been a total of 58 studies related to effects of glucose on postprandial BP in a range of cohorts including health [7-9, 15, 19, 22-24, 36, 40, 44, 45, 50, 54, 70, 74, 75, 83, 88, 90, 104, 106, 109, 111, 124, 126, 133, 134, 139, 140, 144, 158, 161, 197, 359-361, 364-368, 370], PPH [88, 144], diabetes [7, 36, 43, 76, 109, 111, 155, 158, 159, 180, 188, 369], autonomic failure [40, 42-45], PD [43, 50-52], hypertension [19, 79, 362-365], multiple system atrophy (MSA) [43-45, 54] and other conditions [33, 42, 79, 126, 134, 367] (Table 5.1). Intensive care unit (ICU) survivors are more predisposed to PPH; a marked and prolonged decrease in SBP and diastolic BP (DBP) was observed in 35 older patients discharged from the ICU [33], 10 of whom

experienced PPH [33]. In healthy older participants [7, 70, 74] and patients with T2D [7, 111], the fall in BP following glucose ingestion has been shown to be related to the rate of gastric emptying of glucose, i.e. faster gastric emptying is associated with a greater fall in BP. In contrast, in healthy young participants, there is little fall, and in some cases an increase, in SBP following oral glucose [79, 370]. The fall in postprandial BP appears to be on a continuum so that with increasing age the fall in BP is greater. In a study by our group, following a 75g glucose drink in 300 mL water, there was a fall in SBP and a rise in HR in both healthy older participants and people with PPH compared with iso-volaemic water [144], however, the maximum postprandial fall in SBP was greater in the participants with PPH [144].

5.4.1.1 Intraduodenal glucose infusion

When the protective mechanism of gastric distension [90, 368] is bypassed by infusing glucose directly into the duodenum, the fall in BP is greater compared to oral glucose in healthy older participants [139]. Vanis et al. showed that gastric distension, by as little as 100 mL water, could mitigate the fall in BP induced by ID glucose in healthy older participants, supporting the potential use of non-nutritive gastric distension in the management of PPH [90]. ID glucose-induced reductions in BP are observed in both older people [15, 88, 139] and patients with T2D [76, 180, 369]. The hypotensive response to ID glucose depends primarily on the small intestinal glucose load, but solution concentration does not appear to be important [75]. Within the normal physiological range of gastric emptying (1-4 kcal/min) [220], the rate of small intestinal delivery correlates with the magnitude of the fall in BP [8, 76, 83]. Trahair et al. reported that SBP decreased significantly during 2 kcal/min and 3 kcal/min glucose infusions, but not during 1 kcal/min glucose infusion; indicating that a threshold for the fall occurs between 1-2 kcal/min [306]. On the contrary, young healthy participants with sufficient

cardiovascular responses remain normotensive [83] or become only slightly hypotensive [88] after ID glucose infusion. There is no difference in postprandial BP following ID glucose infusion between young healthy and obese groups [367].

5.4.1.2 Management of PPH

Due to its ability to induce a prominent hypotensive response, several studies investigating non-pharmacological [22, 23, 90, 158, 159, 368] and pharmacological [19, 43, 45, 106, 111, 126, 161, 180, 197, 362, 363, 369] approaches to the management of PPH have used glucose as test meals. Interventions based on slowing gastric emptying [22, 23] and small intestinal absorption of nutrients [24] attenuate the postprandial fall in BP. Guar gum, a vegetable-based, gel-forming non-absorbable carbohydrate, commonly used as a bulking agent, delays gastric emptying, slows the intestinal absorption of glucose and, accordingly, attenuates the magnitude of the fall in BP after oral and ID glucose [22, 23, 155]. Intermittent walking may also be effective in preventing PPH induced by glucose ingestion [158, 159]. In terms of the pharmacologic management of PPH, numerous medications have been tested. One study has shown that maximal postprandial fall in sitting SBP after a standardised 400 kcal glucose drink is attenuated by caffeine [161], although the benefits of caffeine as a treatment for PPH are inconsistent and largely empirical. The effects of the somatostatin analogue, octreotide, in attenuating the fall in BP after oral glucose has been demonstrated in 3 studies [19, 45, 363]. These effects are not mediated via the inhibition of insulin secretion [363]. Voglibose, an α-glucosidase inhibitor, is effective in attenuating PPH in neurologic patients [126]. More recently, GLP-1 based therapy has drawn more attention. In a placebo-controlled study, intravenous GLP-1 infusion (0.9 pmol kg−1.min−1) in 14 healthy older individuals and 10 patients with T2D, resulted in the slowing of gastric emptying and attenuation in the fall in BP following ingestion of a radiolabelled 75g glucose drink [109]. GLP-1 also reduces the

maximum fall in SBP in response to ID glucose [106]. Both short-acting GLP-1 receptor agonists, exenatide [180] and lixisenatide [111], known to slow gastric emptying, markedly attenuate the decrease in BP compared to placebo. In contrast, the dipeptidyl peptidase-4 (DPP-4) inhibitor, vildagliptin, known to block the degradation of the incretin hormones GLP-1 and GIP, did not attenuate the fall in SBP and DBP during ID glucose administration compared with placebo in T2D [369]. Recent evidence suggests that GIP may have detrimental effects to lower postprandial BP and it is possible that the beneficial effects of GLP-1 by DPP-4 inhibition, were nullified by GIP. The effect of the anti-diabetic medication, metformin, has also been evaluated in two studies [188, 369], but the outcomes are inconsistent and more studies are warranted. Other investigators have reported that the antihypertensive, nitrendipine [362], or a combination of selective α1 and β1-adrenergic agonists [43], might attenuate the postprandial fall in BP after glucose ingestion, while the antiemetic drug, granisetron, a 5-HT3 antagonist, [197] has no benefit in the management of PPH.

5.4.2 Fructose

Fructose, a monosaccharide found in fruit, honey, and some vegetables [340], may be preferred over glucose by consumers and cooks due to its intrinsically greater sweetness and ability to improve the appearance and texture of baked goods [371]. Fructose has been used increasingly as an alternative to glucose or sucrose [372-374] in several processed foods and beverages, especially in diets targeting patients with T2D, as it provides fewer calories for the same level of sweetness as glucose [375, 376]. In contrast to glucose, the metabolism of fructose primarily occurs in the liver, entering cells in an insulin-independent fashion [377]. Fructose is not an insulin secretagogue [377], inducing a substantially lower glycaemic response compared with glucose [374]. However, although there is no clear consensus in the literature, the potential for

increased de novo lipogenesis from excessive fructose intake, particularly in those with insulin resistance, has raised the concern regarding the widespread use of this monosaccharide [378]. Compared with glucose, fructose is absorbed more slowly from the intestine [379, 380]. There has been a total of 5 studies relating to the effects of oral fructose on postprandial BP in a range of cohorts including in healthy participants [133, 134, 359, 360, 370] and hypertensive patients [134] (Table 5.2).

Some studies have demonstrated no change in BP in healthy young [134] and older participants [133] as well as those with hypertension [134] following ingestion of fructose. In healthy older people, SBP decreased significantly from baseline following glucose: -3.96±1.38mmHg but not after the fructose drink: 2.59±1.62 mmHg [134] (Figure 5.3). Two other studies reported an increase in BP in healthy young participants following ingestion of fructose [359, 360]. This may be due to the slower intestinal absorption rate of fructose and lower glycaemic response compared with glucose [381, 382], which may also result in a limited increase in SMA blood flow. However, there have been no studies comparing the difference in postprandial intestinal vascular perfusion following oral glucose and fructose. In addition, due to the lower insulinotropic effects of fructose compared with glucose and sucrose [383], effects on total vascular peripheral resistance may be mitigated [359, 370].

5.4.3 Sucrose

Sucrose is a common table sugar produced naturally in fruit and vegetables and often added to many processed foods [384]. Sucrose tastes sweeter than glucose, but not as sweet as fructose. The digestion of sucrose is initiated in the mouth and stomach, but the majority of this disaccharide is hydrolysed and digested in the small intestine by the α-glucosidase sucrase to release an equimolar mixture of glucose and fructose [385]. There have been 5 studies relating

to effects of sucrose on postprandial BP in healthy participants [18, 107, 133, 360, 386] (Table 5.2): 4 used an oral load [18, 133, 360, 386] while one study used an ID infusion [107].

The cardiovascular effects of sucrose are more similar to those observed following glucose than fructose, despite its equimolar constitution [387]. Visvanathan et al. reported that ingestion of 50g sucrose or 50g glucose-induced a comparable fall in SBP in healthy elderly individuals, but the decrease in SBP occurred earlier after glucose, than sucrose, ingestion [133] (Figure 5.3). This may be due to the fact that sucrose is a disaccharide, requiring hydrolysis prior to mediating cardiovascular effects [133]. In comparison, a 60g sucrose drink had no substantial impact on BP in healthy young participants [360], which may be due to the more effective postprandial haemodynamic responses compared with an older population. The α-glucosidase inhibitor, acarbose, is approved for the treatment of T2D and is known to reduce postprandial glycaemia [164] through stimulation of GLP-1 [168], and slowing of gastric emptying [18, 162]. In healthy older participants, acarbose substantially decreases falls in SBP and DBP induced by not both oral [18], and ID [107] sucrose, loads.

5.4.4 D-Xylose and Xylitol

Xylose, a monosaccharide of the aldopentose type consists of five carbon atoms and is derived from wood. The free aldehyde group reduces its sweetness and makes it a widely used alternative for glucose in the diabetic diet. Xylose is primarily absorbed by passive penetration through the wall of human duodenum and jejunum [388], with the remainder delivered to the ileum and the colon. Xylose is emptied from the stomach at a similar rate to glucose [104]. There have been 3 studies related to effects of oral xylose on postprandial BP in healthy older participants [40, 42, 104], orthostatic hypotension (OH) [42] and autonomic failure [40, 42] patients (Table 5.2). While Mathias et al. [40] reported xylose induced a milder and more

transient fall in postprandial BP compared with glucose in those with chronic autonomic failure, Robinson et al. reported that glucose and xylose ingestion induced a comparable fall in SBP at 60-90 min in supine patients diagnosed with OH and autonomic failure [42]. In a previous study by our group, designed to compare effects of glucose and xylose on BP, gastric emptying and incretin hormones, xylose emptied from the stomach at a comparable rate to glucose, with a greater and more prolonged effect on GLP-1 secretion in healthy older participants [104]. However, there was no change in postprandial BP observed, potentially due to the effects of GLP-1 in delaying the nutrient absorption in the distal small intestine. These findings support the replacement of glucose by xylose as a simple management to decrease the postprandial fall in BP [104].

Reduction of xylose by catalytic hydrogenation produces xylitol [389]. Xylitol has a comparable sweetness to sucrose and contains less than one-third of calories in conventional sugars. As xylitol is metabolised in an insulin-independent manner, its use has been widely advocated in the prevention and control of hyperglycaemia, obesity, and related metabolic disorders [390, 391]. However, xylitol is often associated with diarrhoea due to its stimulatory effect on the intestinal transit and the release of plasma motilin [392]. Salminen et al. [392] reported that 30g xylitol solution was emptied from the stomach at a significantly slower rate than a 30g of glucose, suggesting that xylitol might protect against PPH, although further studies are required.

5.4.5 Erythritol

Erythritol, a four-carbon pentose, is made through food fermentation but may occur naturally in a wide variety of fruits and mushrooms. Erythritol has a sweetness of ~70% compared with sucrose and has minimal insulinotropic effects [393, 394]. Nevertheless, the effect erythritol

on BP has not been studied. In patients with T2D, acute consumption of erythritol has vasoprotective effects by decreasing oxidative stress and endothelial dysfunction [395].

5.4.6 Maltose and maltodextrin

Maltose, or malt sugar, a disaccharide composed of two glucose units, is an intermediate in the intestinal breakdown of glycogen and starch and is found in germinating grains [396]. Maltodextrin is a nutritive saccharide polymer consisting of D-glucose units with low sweetness (dextrose equivalency of less than 20). It is often used in the production of soft drinks, candy, and processed food [397]. Both maltose [398] and maltodextrin [399] are emptied more slowly from the stomach compared with sucrose, although there is no significant difference in gastric emptying between maltodextrin and glucose [399]. To our knowledge, there have been no studies investigating the effects of maltose on postprandial BP. There has been only one study related to effects of oral maltodextrin on postprandial BP in healthy older participants [386] (Table 5.2) in which postprandial MAP and DBP were shown to be greater after sucrose compared to after maltodextrin [386]. The underlying mechanisms remain unclear [386].

5.4.7 Tagatose

Tagatose has a very similar structure to sucrose, is commonly found in dairy products [400] and is low calorie; only 20 to 25% of ingested D-tagatose is absorbed through the small intestine to provide 1.5kcal/g [401]. The remaining ingested tagatose is fermented into short-chain fatty acids by colonic bacteria [340, 402]. Tagatose has a substantial hypoglycaemic effect without major adverse effects, contributing to its potential therapeutic benefits in obese people and patients with T2D [403-406]. While tagatose has been shown to slow gastric

emptying [407, 408], to our knowledge there is no information regarding the effect of tagatose on BP.

5.5 Non-nutritive sweeteners

5.5.1 Sucralose

Sucralose, a noncaloric artificial sweetener with a sweetness approximately 600 times higher than that of sucrose [409], is increasingly being used as a sugar substitute to reduce calorie intake, especially in obese people and those with diabetes [341]. It is almost unabsorbed in the small intestine and excreted in the faeces in both animal [410, 411] and human [412] experiments. In healthy participants, sucralose empties at a similar rate to saline [413].

There have been 2 studies investigating effects of sucralose on postprandial BP [366, 414] (Table 5.3). Kazmi et al. reported that there was no change in BP following oral sucralose in 200 young healthy participants compared to saline. In a recent study by our group, while ID infusion of glucose at a rate of 3 kcal/min induced a substantial fall in postprandial BP and increase in SMA blood flow in 12 healthy older participants, there were no significant effects on BP or splanchnic blood flow following an ID infusion of sucralose [366] (Figure 5.4). Studies by others have shown that sucralose has no effect on insulin, GLP-1 or GIP secretion or SMA blood flow [413, 415], supporting a therapeutic role for sucralose in the dietary management of PPH.

5.5.2 Acesulfame-K

Acesulfame-K, a non-nutritive sweetener, was invented in 1967 by the pharmaceutical company, Hoechst AG. It is estimated to be 200 times sweeter than sucrose and is often mixed

with sucralose or aspartame in foods and beverages [349]. In 2009, Brown et al. reported that the GLP-1 release increased by more than 30% after drinking "diet soda", comprising sucralose, aspartame and Acesulfame-K compared to carbonated water [416]. However, this finding was not replicated in subsequent studies [415, 417]. Acesulfame-K alone or combined has no effect on GLP-1 secretion, insulin, or blood glucose. There has been only one study investigating the effect of Acesulfame-K on BP [414] (Table 5.3). Kazmi et al. reported that SBP was lower at 60 min following the ingestion of 3.24gm (45 mg/kg) of Acesulfame-K in 50 healthy young participants compared to controls. The design of that study was, however, suboptimal, as SBP and DBP were only measured at 30, 60, 90 and 120 min and the baseline measurement was not taken into account.

5.5.3 Aspartame

Aspartame, a methyl ester of aspartic acid and phenylalanine dipeptide, was approved as a non-nutritive sweetener in 1996 by the FDA. It is 160 to 220 times sweeter than sucrose and the only non-nutritive sweetener providing energy (4 kcal/g). However, due to the intense sweetness, only a tiny quantity is used in foods and soft drinks and the number of calories is negligible, hence, it is considered 'non-nutritive' [340, 341]. Little et al. [418] demonstrated that the gastric emptying of aspartame does not differ from that of water. There has been only one study investigating the effect of aspartame on BP [414] (Table 5.3). In this study, young healthy participants experienced falls in SBP at 60, 90 and 120 min following the ingestion of 10.8 g (150mg/kg) aspartame compared to 10 g of cellulose (control) (109.82 ± 11.39 vs. 116.92 ± 8.98, 112.54 ± 11.18 vs. 117.84 ± 10.2 and 112.02 ± 10.54 vs. 117.16 ± 8.62 respectively) [414]. Again, the design of that study was, suboptimal, as SBP and DBP were only measured at 30, 60, 90 and 120 min and the baseline measurement was not taken into account.

5.5.4 Saccharin

Saccharin is the oldest non-nutritive sweetener that is currently approved, is not metabolised in humans and is considered non-carcinogenic [340, 341]. To date, there are no studies investigating the acute effects of saccharin on BP.

5.5.5 Steviol glycoside

Steviol glycoside sweeteners are extracted and purified from the leaves of the Stevia rebaudiana Bertoni plant, which is native to South America. Its sweet potency is reported to be 200 to 400 times sweeter than table sugar [419, 420]. Steviol glycoside has been categorised as a non-caloric sweetener and widely used in the food and soft drinks by millions of people and is well tolerated [400]. In one study, postprandial blood glucose and insulin levels were significantly lower when healthy lean and obese participants consumed a steviol glycoside preload before meals compared with an isocaloric sucrose preload [421]. Another study in participants with T2D reported that steviol glycoside reduced postprandial blood glucose without a significant change in insulin levels [422]. Furthermore, steviol glycoside was also shown to significantly reduce food intake [421]. Peirera et al [423] did not demonstrate any effect of steviol glycoside on gastric emptying of a liquid test meal in 10 male healthy volunteers. Most of the studies in the literature have evaluated the effect of long-term steviol glycoside intake on BP and demonstrated that it may be effective in decreasing SBP and DBP compared with placebo in hypertensive [424, 425], but not in normotensive or hypotensive, participants [426, 427]. However, there have been no publications evaluating the acute effect of this sweetener on postprandial BP.

5.5.6 Neotame and advantame

Neotame is closely related to aspartame in terms of the chemical constitution. It is 7000–13,000 times sweeter than sucrose [428]. Advantame is considered the most potent non-caloric sweetener with sweetness approximately 20,000 times greater than sucrose [428]. Advantame is derived from isovanillin and aspartame [429]. To our knowledge, there have been no studies evaluating the effects of these sweeteners on BP.

5.6. Conclusions

PPH is associated with an increased incidence of falls, syncope, angina and transient ischaemic attacks, particularly in older people and patients with autonomic failure that is frequently secondary to diabetes mellitus and PD. For nutritive sweeteners, glucose induces the greatest fall in postprandial BP and should be limited in people with PPH. Most of the currently available literature indicates that non-nutritive sweeteners have little effect on BP. While pharmacological and dietary approaches are being explored, current management of PPH remains suboptimal. This literature review supports the view that low nutritive (D-xylose, xylitol, erythritol, maltose, maltodextrin, and tagatose) and non-nutritive sweeteners could be used to replace high nutritive sweeteners (glucose, fructose, and sucrose) in the dietary management of PPH.

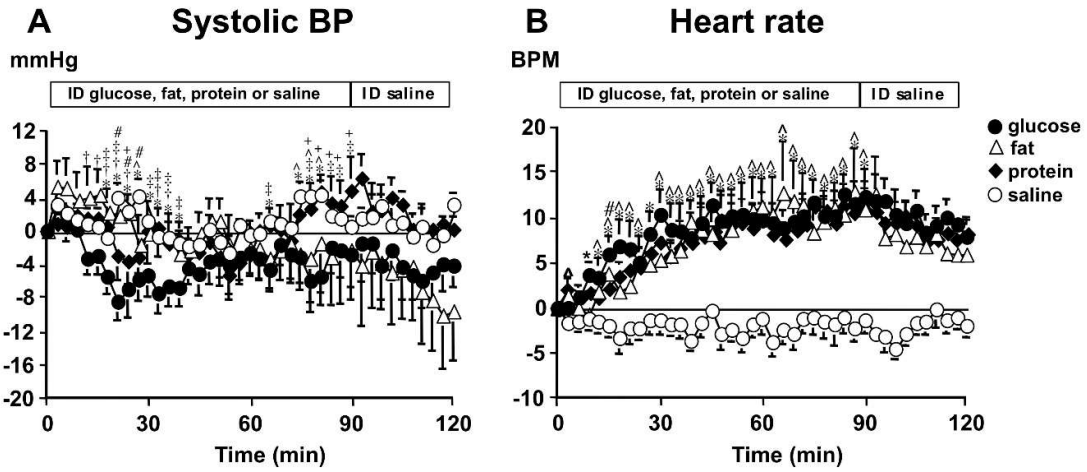

Figure 5.1. Changes from baseline in (A) Systolic blood pressure (BP) and (B) Heart rate in 8 healthy older participants in response to intraduodenal (ID) infusion of glucose, fat, protein, and saline. Data are mean values ± SEM. *P < 0.05 for glucose compared with saline; †P < 0.05 for glucose compared with fat; ‡P < 0.0001 for glucose compared with protein; ^P < 0.05 for fat compared with saline; #P < 0.05 for protein compared with saline; +P < 0.05 for fat compared with protein [9].

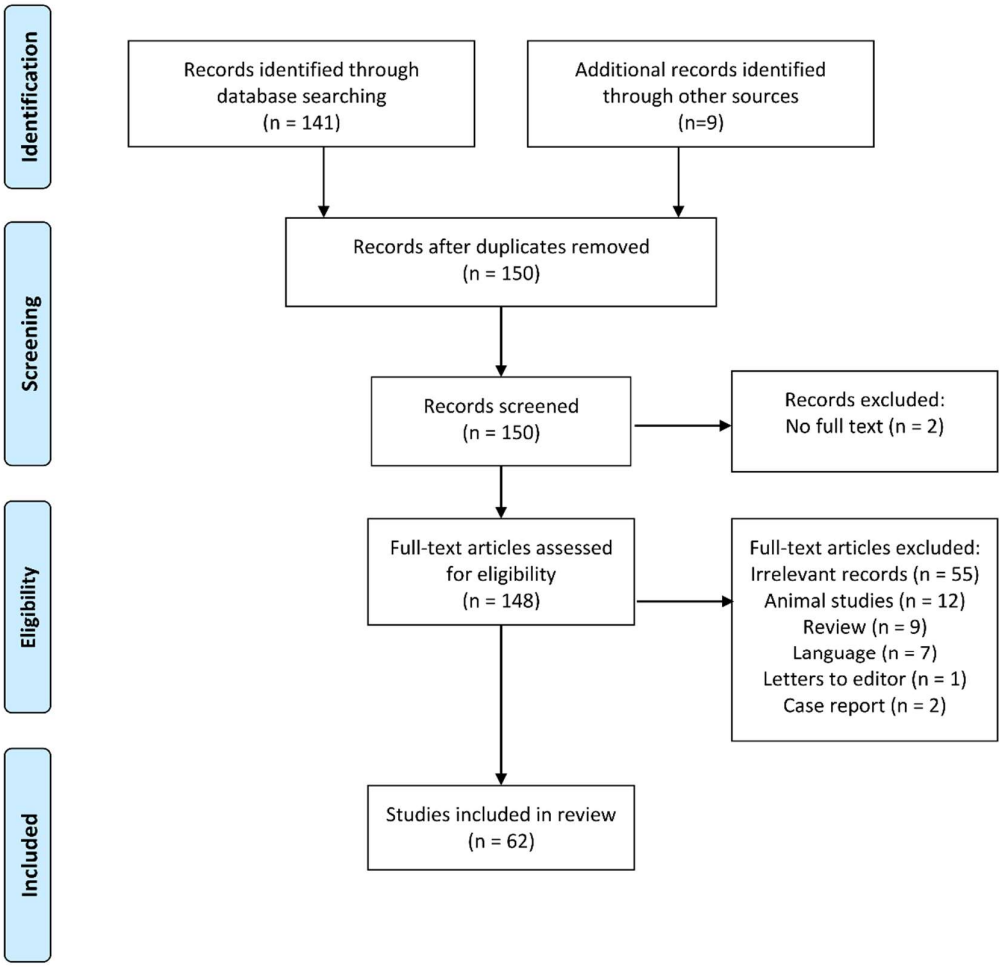

Figure 5.2. Flow diagram for the selection of studies for review based on the preferred reporting items for systematic reviews and meta-analyses (PRISMA) 2009 statement.

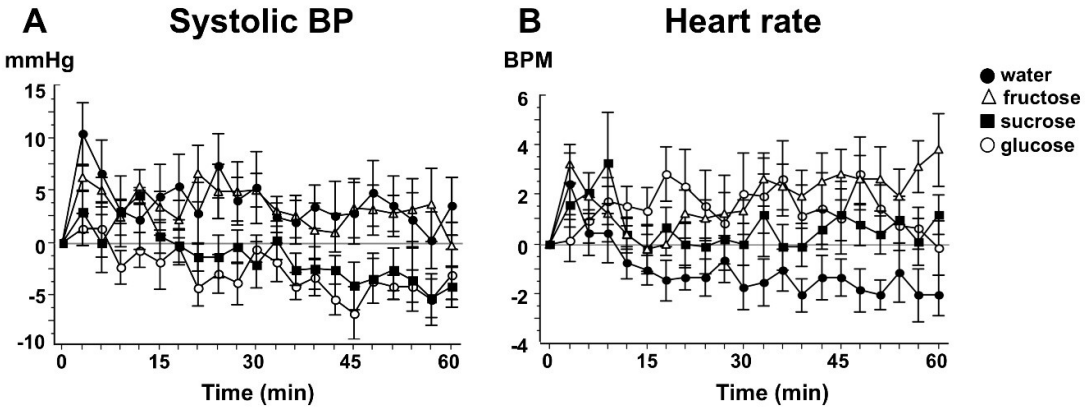

Figure 5.3. Change from baseline in (A) Systolic blood pressure (BP) and (B) Heart rate in 10 healthy older people following ingestion of four different study drinks (○, glucose; ■, sucrose; Δ, fructose; ●, water). Data are mean values ± SEM [133].

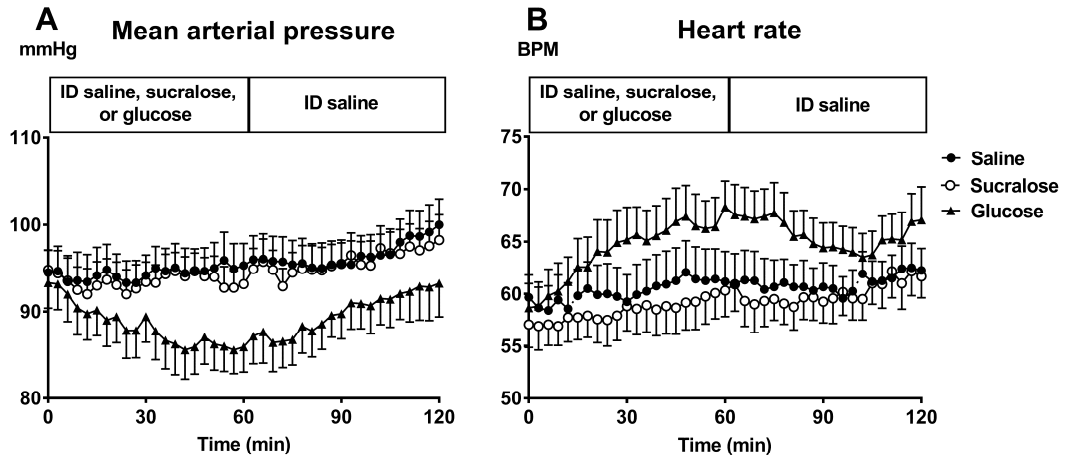

Figure 5.4. (A) Mean arterial pressure (MAP) and (B) Heart Rate in 12 healthy older subjects after intraduodenal (ID) infusion of glucose (▲), sucralose (o), and saline (•). Data are mean values ± SEM. MAP: $P < 0.05$ for glucose compared with sucralose or saline, and $P = 1.0$ for sucralose compared with saline. Heart rate: $P < 0.05$ for glucose compared with sucralose, $P = 0.07$ for glucose compared with saline, and $P = 1.0$ for sucralose compared with saline [366].

Table 5.1. Studies relating to the effects of glucose on blood pressure

Study	Year	Participant characteristics	Study Design	Test meal	Effects on blood pressure
Borg et al. [188]	2019	10 diet controlled T2D patients, 5M:5F, aged 65.6 ± 3.1 years	Randomised crossover study	ID metformin (1g) or saline (control) 60 minutes before ingesting a 50g glucose drink labelled with 150 mg 13C-acetate.	SBP and DBP decreased following oral glucose on both days. The fall in SBP was less after metformin than control.
Brown et al. [359]	2008	15 healthy normal-weight participants, 9M:6F, aged 24 ± 1 years	Randomised crossover study	500 mL of either water, 60g glucose, or 60g fructose.	Oral fructose, but not glucose, significantly increased SBP and DBP. The maximum rise in SBP after fructose was 6.2 ± 0.8 mmHg.
Charriere et al. [370]	2017	9 young healthy men, aged 24 ± 1 years	Randomised crossover study	500 mL of water containing 60g of either glucose, fructose or galactose.	The increase in SBP after fructose (7–8 mmHg) was greater than after glucose (4–5 mmHg) or galactose (2–3 mmHg). DBP increased to a greater extent with fructose (~5 mmHg), compared to non-significant increases of only 2–3 mmHg after glucose or galactose.
Edwards et al [124]	1996	10 young (20-40 years), 9 middle-aged (41-50 years), and 10 old (61-83 years) participants	Non-randomised study	75g glucose in 300 mL water	SBP decrease was significant in both the older groups. A fall in SBP >15mmHg observed in 5 individuals; 4 aged > 60 years and 1 middle-aged.
Fagius et al. [361]	1994	39 participants in 5 groups: glucose (n = 8, 4M:4F, mean age 25.8 years), fat (n = 8, 5M:3F, mean age 25.5 years), protein (n = 8, 5M:3F, mean age 25.6 years), mixed meal (n = 8, 6M:2F, mean age 26.2 years) or water (n = 7, 4M:3F, mean age 24.9 years).	Parallel study	100g glucose in 300 mL of water (n = 8), 50g fat in 250 mL of water (n = 8), 100g lean meat (40g protein) with 250 mL water (n = 8), 300 mL water alone (n = 7) or a mixed meal (n = 8).	Small and sometimes significant increases in BP occurred during the sessions.
Fagius et al. [79]	1996	3 groups - A: 9 young healthy, 5M:4F, aged 26.2 ± 2.8 years; B: 9 older healthy, all M, aged 73.0 ± 0.7 years; C: 9M with insulin resistance aged 72.8 ± 1.6 years	Non-randomised study	100g D-glucose in 300 mL	Significant fall in BP observed in groups B and C but not in group A, who demonstrated an increase in SBP.
Gentilcore et al. [75]	2006	8 healthy older participants, 3M;5F, aged 65-78 years	Randomised crossover study	50g glucose in either 300 mL (16.7%), 600 mL (8.3%), or 1200 mL (4.1%) or saline (0.9%) at a similar rate of 3 kcal/min	SBP and DBP decreased, and HR increased, on all days following the glucose infusions with no difference between them.

Gentilcore et al. [197]	2007	10 healthy older participants, 5M:5F, aged 65-76 years	Randomised crossover study	Granisetron (10mcg/kg) or control (saline) at t = -25min; ID glucose infusion (3 kcal/min) for 60min, followed by ID saline for a further 60min.	There were falls in SBP and DBP and a rise in HR during ID glucose; granisetron had no effect on these responses.
Gentilcore et al. [9]	2008	8 healthy older participants, 4M:4F, aged 68–79 years	Randomised crossover study	ID glucose (64g), fat (10% oil emulsion), protein (72g whey), or saline (0.9%) infusion at a rate of 2.7 mL/min for 90min, followed by ID saline for 30min	The maximum falls in SBP during the glucose (11.7 ± 2.8 mmHg), fat (11.7 ± 4.8 mmHg), and protein (11.0 ± 1.5 mmHg) infusion did not differ significantly. The fall occurred significantly earlier during the glucose (18 ± 3 min) than during the fat (46 ± 11 min) and protein (33 ± 7 min) infusion.
Gentilcore et al. [15]	2008	8 healthy older participants, 5M:3F, aged 65-76 years	Randomised crossover study	(1) ID glucose (50g) or (2) ID glucose (50g) with intragastric infusion of 500 mL water or (3) ID saline (0.9%) with intragastric infusion of 500 mL water.	The fall in SBP and DBP greater during (1) and (2) when compared with (3) and (1) compared with (2). Gastric distension attenuated the fall in BP.
Gentilcore et al. [139]	2009	8 healthy participants, 5M:3F, aged 66-75 years	Randomised crossover study	Day 1: ingestion of 75g glucose in 300 mL. Gastric emptying rate (kcal/min) quantified by 3D ultrasound between t = 0-120 min. Day 2, ID glucose infused at the same rate as day 1.	SBP was greater less after oral, compared with ID glucose.
Grasser et al. [360]	2014	12 healthy young adults, 7M:5F, aged 22.0 ± 0.4 years	Randomised crossover study	500 mL drink of either 60g sucrose, 60g glucose, 60g fructose or 30g fructose.	Ingestion of fructose (60 or 30 g) elevated SBP, DBP and mean arterial pressure (MAP). Ingestion of glucose elevated SBP. Ingestion of sucrose showed no BP changes. The increases in DBP and MAP were significantly higher for fructose (60 or 30 g) than for either glucose or sucrose. The increase in SBP was significantly higher for fructose than for sucrose.
Heseltine et al. [161]	1991	20 older adults, 10M:10F, aged 84 ± 5 years	Randomised crossover study	400kcal glucose drink with either caffeinated coffee or decaffeinated coffee	Maximal postprandial fall in sitting SBP was attenuated by caffeine. Four participants developed symptomatic PPH after placebo which was prevented by caffeine.
Hirayama et al. [44]	1993	10 patients with MSA, 9M:1F, aged 57 ± 7 years, 3 patients with peripheral autonomic neuropathy, 2M,1F, aged	Non-randomised study	75g glucose in 225 mL water	In MSA, ingestion of glucose resulted in a rapid and significant fall of SBP and DBP. In peripheral autonomic neuropathy, BP

		35-57 years and 16 controls, 14M:2F, aged 38 ± 11 years.			decreased within 15 min of oral glucose ingestion, but soon recovered. BP in the controls remained unchanged.
Hirayama et al. [43]	1993	5 patients with MSA, 3M:2F, aged 50-71 years, 2 patients with pure autonomic failure, 2M: 54-78 years and 1 71-year-old F patient with autonomic failure and Parkinson's disease. All with PPH and OH	Crossover study	Denopamine and midodrine administered 30 min before 75g glucose drink on one day versus no drug a few days prior.	PPH was prevented by denopamine and midodrine.
Hoeldtke et al. [45]	1989	6 MSA patients, 4M:2F, aged 53-73 years, 5 progressive autonomic failure patients, 3M:2F, aged 41-84 years) and 14 controls, 9M:2F, aged 36-89 years.	Crossover study	SMS-201-995 (0.8mcg/kg) or placebo injection sc before consuming a 50g glucose drink.	In patients with progressive autonomic failure and MSA, glucose ingestion caused a decrease in BP which was attenuated by SMS-201-995.
Jansen et al. [134]	1987	10 young normotensive people, aged 28 ± 1 years (YN), 10 young hypertensive patients, aged 44 ± 2 years (YH), 10 elderly normotensive people aged 75 ± 2 years (EN), 10 elderly hypertensive patients aged 75 ± 1 years (EH).	Randomised crossover study	300 mL drink of either 75g glucose or 75g fructose.	Glucose decreased MAP significantly in the EH, EN and YH group. After fructose, BP remained unchanged in 4 groups.
Jansen et al. [362]	1988	Hypertensive patients: randomised to nitrendipine: 4M:5F, aged 70-78 years – or hydrochlorothiazide: 3M:10F, aged 70-84 years	Randomised parallel study	75g glucose drink before and after treatment with 20mg nitrendipine once daily or 50mg hydrochlorothiazide once daily for 12 weeks.	After 12 weeks of treatment, nitrendipine reduced the fall in MAP after oral glucose (6%, P< 0.01) but this was not significant for hydrochlorothiazide (4%, NS).
Jansen et al. [19]	1989	10 hypertensive participants, 3M:7F, aged 74 ± 4 years; and 10 normotensive participants, 4M:6F, aged 74 ± 4 years	Randomised crossover study	Octreotide (50mcg sc) or placebo (154mmol/l NaCl) before oral 75g glucose in 300 mL water	Octreotide attenuated the fall in MAP (15 ± 1 mmHg in the 10 hypertensive participants and 7 ± 2 mmHg in the 10 normotensive participants) induced by oral glucose.
Jansen et al. [363]	1989	10 hypertensive participants, 7M:3F, aged 73 ± 3 years	Randomised crossover study	Octreotide (50mcg sc) at t = −30 min followed by placebo or insulin (0.3U/kg) sc at t = −10 min and oral glucose (75g in 300 mL water) at t = 0 minutes	The fall in MAP after oral glucose was attenuated by octreotide with no difference between the insulin and placebo study days.
Jansen et al. [364]	1989	15 older hypertensives (EH), 7M:8F, age 73 ± 3 years, 15 older normotensives (EN), 6M:6F, age 76 ±	Non-randomised study	75g glucose in 300 mL water	In both elderly groups MAP decreased significantly after the glucose load, whereas no

		4 years and 10 young normotensives (YN), 5M:5F, age 26 ± 4 years.			change was observed in the YN. Glucose load did not influence baroreflex sensitivity.
Jones et al. [7]	1998	16 T2D patients, 11M:5F, aged 39–79 years; 10 young healthy participants, 9M:9F, aged 19–26 years; 9 older healthy participants, 6M:3F, aged 40–68 years old.	Non-randomised study	75g glucose in 350 mL water	The fall in MAP was significantly greater in the T2D than in older healthy participants with no change in young healthy participants. The magnitude of the fall in BP was related to the rate of gastric emptying.
Jones et al. [140]	2005	10 healthy participants, 6M:6F, aged 73.9 ± 1.2 years	Randomised crossover study	25g glucose in 200 mL (12.5%), 75g glucose in 200 mL (37.5%), 25g glucose in 600 mL (4%), and 75g glucose in 600 mL (12.5%)	Increased drink volume attenuates the fall in BP with no effect of glucose concentration.
Jones et al. [22]	2001	10 healthy participants, 5M:5F, aged 67 -78 years	Randomised crossover study	300 mL water containing 50g glucose with 30 mL lemon juice made up to 300 mL with or without 9g guar gum	SBP, DBP and MAP fell on both days. The magnitude of the falls in SBP, DBP, and MAP were less, after guar.
Jones et al. [111]	2019	15 healthy participants, 9M:6F, aged 67.2 ± 2.3 years and 15 T2D patients, 9M:6F, aged 61.9 ± 2.3 years)	Randomised crossover study	Lixisenatide (10 mcg) or placebo sc 30 min before 75g glucose drink on two separate days.	Lixisenatide attenuated the decrease in SBP and DBP compared to placebo in healthy participants and those with T2D
Marathe et al. [76]	2016	9 patients with T2D, all M, aged 62 ± 2.4 years	Randomised crossover study	ID glucose (25g/100 mL) infused at 2 kcal/min or 4 kcal/min	SBP and DBP fell at 30min with 4 kcal/min, but not 2kcal/min infusions. The fall in SBP was greater after the 4kcal/min infusion.
Maruta et al. [76]	2006	28 neurologic patients (11 with PD, 4M:7F, aged 61-86 years; 6 with MSA, 4M:2F, aged 53-76 years; 11 with T2D, 8M:3F, aged 62-85 years) and 20 healthy controls (13 older participants, 5M:8F, aged 62-80 years; 7 young participants, 4M:3F, aged 34-59 years).	Crossover study	75g glucose with or without 200 mcg voglibose. All participants were studied on the day without voglibose. 11 of them (4 with PD, 5 with MSA, 1 with T2D, 1 older control), who had PPH, were studied on the day with voglibose.	The fall in BP was less (without voglibose: 41.5 ± 13.2 mmHg, with voglibose: 21.0 ± 13.0 mmHg) and the duration of PPH was shorter (without voglibose: 52.3 ± 28.0 minutes, with voglibose: 17.3 ± 22.5 minutes) after voglibose.
Masuo et al. [365]	1996	12 young normotensive (NT) participants, aged 47.8 ± 2.6 years; 21 elderly NT, aged 77.9 ± 1.5 years; 17 young hypertensive (EH) patients, aged 49.0 ± 1.9 years and 32 elderly EH. 1M:1F in each group.	Non-randomised study	75g glucose in 225 mL water	Postprandial BP reduction, defined as 10% or more decline in MAP was recognised in 3/12 (25%) young NT, 9/21 (43%) elderly NT, 5/17 (29%) young EH, and 20/32 (63%) elderly EH. The frequency of postprandial BP reduction was significantly greater in elderly hypertensives compared to elderly normotensives and was greater in young hypertensives compared to young normotensives.

Mathias et al. [40]	1989	6 patients with chronic autonomic failure (CAF), 4M:2F, aged 42-68 years, 6 age-matched participants without CAF, aged 45-70 years; and 8 normal participants, all M, aged 28-35 years.	Randomised parallel study	An iso-osmotic solution of glucose (1g/kg body weight) or xylose (0.83g/kg body weight) in 250 mL water. 6 patients with CAF attended 2 on both glucose and xylose days. 6 age-matched participants and 8 male normal participants attended only on the glucose day.	Xylose caused a lower and more transient fall in BP than glucose in patients with CAF (15 ± 6% vs. 34 ± 7%). After glucose, there was a substantial fall in 6 age-matched participants but a minimal change in 8 male normal participants.
Nair et al. [159]	2015	13 participants with PPH, 4M:9F, aged 76.5 ± 4 years.	Randomised crossover study	Ingestion of 50g glucose in 200 mL on both days. On one day, participants walked at their usual pace for 30 m every 30 minutes for 120 minutes.	On the control day, there were significant falls in SBP and DBP. On the intervention day, there was no significant fall in SBP, however, DBP still fell significantly.
Nair et al. [158]	2016	29 older participants,18F aged 77.1 ± 5.4 years and 11M aged 74.7 ± 3.9 years	Randomised crossover study	3 treatments: glucose (50g in 200 mL) (G) or water (200 mL) and intermittent walking (WW) or glucose and walking (GW)	16 participants had PPH. In PPH, there was a significant fall in SBP (26.69 ± 8.43 mmHg) on the "G" day and no change on "GW" or "WW" days. In those without PPH, there were no changes in SBP on the "G" or "GW" days, with an increase in SBP on the "WW" day.
Nguyen et al. [33]	2018	35 older participants, 28M:7F, aged 73 ± 5 years, discharged at least 3 months from ICU	Non-randomised study	300 mL drink containing 75g glucose	There were significant reductions in both SBP and DBP. Ten participants (29%) had postprandial hypotension. The maximal fall in SBP and DBP were −29 ± 14 mmHg and −18 ± 7 mmHg. The maximal fall in SBP was greater in patients with PPH than in those without (−46.2 ± 10.8 mmHg vs −22.7 ± 9.2 mmHg).
O'Donovan et al. [8]	2002	8 healthy elderly participants, 4M:4F, aged 70.3 ± 3.4 years	Randomised crossover study	25% glucose solution was infused intraduodenally at a rate of either 1 or 3 kcal/min for 60min followed by 0.9 % saline for a further 60min	Between t = 0-60min, there was a fall in SBP, DBP and MAP during the 3 kcal/min glucose infusion, but not during the 1 kcal/min infusion.
O'Donovan et al. [23]	2005	8 healthy older participants, 4M:4F, aged 70.3 ± 3.4 years	Randomised crossover study	ID glucose infusion (3 kcal/min) with or without guar gum (4g) for 60min, followed by 0.9% saline intraduodenally for a further 60min.	Between t = 0-60min, SBP was lower during the glucose-only infusion than during the glucose and guar infusion. The maximum fall in SBP on the glucose-only study was 10 ± 4 mmHg. Between t = 0-30min, DBP fell during the glucose-only infusion, but did not change with the glucose and guar infusion.

Pham et al. [366]	2018	12 healthy participants, 6M:6F, aged 73.2 ± 1.1 years	Randomised crossover study	ID infusion of either glucose (25%, ~1400 mOsmol/L), sucralose (4 mmol/L, ~300 mOsmol/L) or saline (0.9%, ~300 mOsmol/L) at a rate of 3 mL/min for 60min followed by ID saline for a further 60min.	MAP decreased during glucose but not during sucralose or saline. By t = 60 min, MAP was lower after glucose (85.9 ± 2.8 mmHg) than after sucralose (93.1 ± 2.2 mmHg) infusions without significant difference between sucralose and saline infusions.
Pham et al. [70]	2019	33 healthy older participants, 16M:17F, aged 77.0 ± 0.7 years	Non-randomised study longitudinal study	75g glucose in 300 mL water	The prevalence of PPH doubled from 9.1% to 18.2%. There was a fall in SBP and DBP on both study days. The AUC of SBP was greater at follow-up. The maximum fall in postprandial SBP between t = 60–120 min was significantly greater at follow-up (−11.7 ± 1.4 vs −15.2 ± 1.6 mmHg).
Robinson et al. [42]	1992	5 participants with age-related OH, 2M:3F, aged 73-88 years), 3 participants with autonomic failure, 1M:2F, aged 72-79 years and 5 controls, 2M:3F, aged 72-86 years	Randomised crossover study	50g glucose or 42g xylose in 100 mL water	In OH and autonomic failure groups, the SBP decreased comparably following glucose and xylose, DBP was lowered 60-90 min after glucose. No significant BP changes in the control group.
Russo et al. [155]	2003	11 patients with T2D managed by diet alone, 8M:3F, aged 61.9 ± 1.3 years	Randomised crossover study	50g glucose and 30 mL lemon juice, with or without 9g guar gum in 300 mL.	There was significant fall in SBP between baseline and 30min on the control day (143.9 ± 4.7 mmHg vs 139.0 ± 4.2 mmHg; P < 0.01), but not after guar (145.1 ± 4.8 mmHg vs 142.6 ± 4.5 mmHg, P = 0.6). There were significant falls in DBP and MAP between baseline and 30min on both study days.
Sasaki et al. [36]	1992	15 normal participants, aged 25-63 years and 35 outpatients with T2D, aged 28-60 years	Non-randomised study	Daily meals and 75g glucose in 300 mL water	No significant change in BP in the normal participants. The incidence of PPH in diabetics was 37% after daily meals and 20% after 75g glucose.
Takamori et al. [54]	2007	17 MSA patients, 9M:8F, aged 59.8 ± 7.9 years and 8 healthy controls, 7M:1F, aged 60.5 ± 8.3 years	Non-randomised study	75g of glucose in 225 mL of water	Of 17 MSA patients, 9 had PPH. 8 controls were PPH negative. The falls in SBP and DBP in MSA with PPH were significantly greater than in MSA without PPH or in controls.
Thazhath et al. [180]	2017	9 patients with T2D, managed by diet alone, 6M:3F, aged 60.7 ± 2.4 years	Randomised crossover study	Intravenous exenatide (7.5mcg) or volume-matched saline control from −30 to 120min + ID glucose (3 kcal /min) from 0–60min.	During the ID glucose infusion, SBP, DBP and MAP increased with exenatide, but fell with saline control. The AUC for DBP and MAP,

					but not SBP, was higher with exenatide than control.
Trahair et al. [109]	2015	14 older healthy participants, 6M:8F, aged 72.1 ± 1.1 years and 10 patients with T2D, 6M: 4F, aged 68.7 ± 3.4 years	Randomised crossover study	Between t = −30-120min: intravenous infusion of GLP-1 (0.9pmol/kg/min) or saline (154 mmol/LNaCl). At t = 0min: 75g glucose drink in 300 mL water	After the glucose drink, there were falls in SBP and DBP in both groups. The fall in DBP in older individuals; and the fall in SBP and DBP in patients with T2D were less after GLP-1 infusion compared to control.
Trahair et al. [83]	2012	12 healthy young participants, 6M;6F, aged 22.2 ± 2.3 years and 12 healthy older participants, 6M; 6F, aged 68.7 ± 1.0 years	Randomised crossover study	ID infusion of glucose at either 1, 2 or 3 kcal/min or 0.9% normal saline for 60min followed by ID saline for a further 60min.	In young participants, there were no changes in SBP and DBP during the four infusions. In older participants, there were falls in SBP and DBP during 2 kcal/min and 3 kcal/min infusions, but not during 1 kcal/min infusion.
Trahair et al. [106]	2014	10 healthy older participants, 9M: 1F, aged 73.2 ± 1.5 years	Randomised crossover study	Between t = -30-60min, intravenous infusion of GLP-1 (0.9pmol/kg/min), or saline for 90. Between t = 0-60min, ID glucose was infused at 3 kcal/min.	During ID glucose infusion, there were falls in SBP and DBP with both GLP-1 and control. The maximum fall in SBP was greater with control than GLP-1 (−13.6 ± 3.1 mmHg vs −8.7 ± 2.3 mmHg).
Trahair et al. [74]	2015	88 healthy older participants, 41M:47F, aged 71.0 ± 0.5 years	Non-randomised study	75g glucose in 300 mL water	SBP and DBP decreased significantly after the glucose drink. Eleven participants (12.8%) had PPH.
Trahair et al. [52]	2016	21 participants with mild to moderate PD, 13M:8F, aged 64.2 ± 1.6 years	Crossover study	75g glucose in 300 mL water	SBP and DBP fell following the glucose drink. 8 participants (38%) had postprandial hypotension.
Trahair et al. [144]	2017	8 healthy older participants, 4M:4F, aged 71.0 ± 1.7 years and 8 participants with PPH 1M:7F, aged 75.5 ± 1.0 years	Randomised crossover study	75g glucose in 300 mL water or water alone	Following the glucose, there were decreases in SBP and DBP in both groups, the maximum fall in SBP was greater in participants with PPH. Following the water, there were no changes in SBP and DBP in healthy participants, but there was a rise in SBP in participants with PPH.
Trahair et al. [367]	2018	12 obese participants, 10M:2F, aged 36.6 ± 3.9 years, BMI: 36.1 ± 1.3 kg/m2) and 23 controls, 16M:7F, aged 27.8 ± 2.4 years, BMI: 22.4 ± 0.5 kg/m2	Randomised crossover study	ID infusions of glucose at 1 or 3 kcal/min, or 0.9% saline, for 60min, followed by saline for a further 60min.	No changes in SBP in both groups during any of the conditions. There was a fall in DBP in controls during 1kcal/min and 3 kcal/min infusions; and in obese participants during 3 kcal/min infusion. There was no difference in BP responses between the groups.

Umehara et al. [50]	2014	37 patients with de novo PD (17 with PPH, 4M:13F, aged 76.8 ± 6.1 years; 20 without PPH, 8M:12F, aged 74.4 ± 7.5 years) and 10 healthy controls, aged 74.3 ± 4.8 years)	Non-randomised study	75g glucose in 300 mL water	Of the 37 patients, 17 (45.9%) had PPH, 15 (40.5%) had OH and 8 (21.6%) had both PPH and OH. 2 controls had PPH. The maximum fall in SBP after the glucose drink significantly correlated with that on head-up tilt-table testing in PD patients.
Umehara et al. [51]	2016	64 de novo patients with PD, 22M:42F, aged 76 ± 4 years	Non-randomised study	75g glucose in 300 mL water	29 patients had PPH. Patients with PPH experienced greater reductions in SBP (30 ± 11 vs 11 ± 15 mmHg) and DBP (14 ± 9 vs 7 ± 5 mmHg) after glucose drink compared to patients without PPH.
van Orshoven et al. [88]	2008	8 healthy young participants (4M:4F, aged 28.8 ± 3.4 years), 8 healthy elderly (4M:4F, aged 75.3 ± 1.6 years). 2 female patients with symptomatic PPH aged 21 and 90 years	Non-randomised study	ID infusion of 25% glucose at 3 mL/min for 60min. Saline was infused for 30min before and after ID glucose.	ID glucose decreased SBP, in both the young and older people, but the fall in SBP was greater in the older group (−6.5 ± 1.6 vs −17.0 ± 4.1 mmHg). 2 PPH patients had a greater fall in SBP than the two healthy groups (-21 and -98 mmHg).
Vanis et al. [24]	2011	12 healthy older participants, 6M:6F, aged 68.7 ± 1.0 years	Randomised crossover study	ID infusion of glucose at either 1, 2 or 3 kcal/min or 0.9% normal saline for 60min, followed by saline for a further 60min.	There was a fall in SBP and DBP during 2 and 3 kcal/min glucose infusions, but not during saline or 1 kcal/min glucose infusion. There was no difference in the maximum falls in SBP during 2 kcal/min (15 ± 2mmHg) and 3 kcal/min (12 ± 2mmHg) loads.
Vanis et al. [104]	2011	8 healthy older participants, 6M:2F, aged 65-75 years	Randomised crossover study	300 mL drink of water, 50g glucose or 50g d-xylose.	There was a fall in SBP after glucose drink and no change after xylose or water drink.
Vanis et al. [368]	2010	8 participants, 6M:2F, aged 65-75 yeas	Randomised crossover study	The four treatments were as follows: ID glucose (3 kcal/min) + barostat (distension) (GD), ID saline + barostat (SD), ID glucose (G), and ID saline (S).	SBP and DBP fell during G, but not during S or GD; and increased during SD. The maximum changes in SBP during G, GD, S and SD were -14 ± 5, -3 ± 4, +11 ± 2, and +15 ± 3 mmHg respectively.
Vanis et al. [90]	2012	9 participants, all M, aged 65-75 years	Randomised crossover study	ID glucose infusion (3 kcal/min) and gastric distension at a volume of 1) 0 mL (V0), 2) 100 mL (V100), 3) 300 mL (V300), or 4) 500 mL (V500).	SBP and DBP fell during V0, but did not change significantly during V100, V300, V500.
Visvanathan et al. [128]	2006	12 elderly participants, 6M:6F, aged 72.2 ± 5.7 years	Randomised crossover study	300 mL drink of either (1) CHO (75g glucose and 93g Polyjoule (CHO polymer)-653 kcal); (2) 88 % fat	SBP decreased following the CHO drink and the high-fat drink but not water; there was no difference in the magnitude of the decrease

				(cream blended with milk-653 kcal) or (3) water.	between the CHO and fat drinks. The onset of the SBP fall was slower after the fat drink (26.5 ± 17.1 min vs 13.0 ± 11.7 min).
Visvanathan et al. [133]	2005	10 healthy older participants, 4M; 6F, aged 72.2 ± 1.50 years	Randomised crossover study	300 mL of either 50g glucose, 50g sucrose, 50g fructose or water + 30 mL lemon juice	SBP decreased significantly following glucose (-3.96 ± 1.38 mmHg) and sucrose (-3.03 ± 1.37mmHg) ingestion, increased non-significantly following fructose ingestion (2.59 ± 1.62 mmHg). The decrease in SBP occurred earlier after glucose than sucrose ingestion (7.33 ± 2.19 vs 21.0 ± 4.30min).
Wu et al. [369]	2017	Study A: 16 participants with T2D, 11M: 5F, 65.5 ± 2.4 years. Study B: 9 participants with T2D, all M, aged 63.8 ± 2.6 years	Randomised crossover study	Study A: vildagliptin (50mg) or placebo was given 60min before ID glucose infusion at 2 or 4 kcal/min (ID2 or ID4). Study B: Participants received metformin (850mg) or placebo for 7 days. On the study day, metformin (850 mg) or placebo was given 30min before ID2.	Study A: SBP and DBP decreased after vildagliptin, but not after placebo, without any difference between ID2 and ID4. Study B: SBP and DBP decreased on both days without any difference between metformin and placebo.

T2D = type 2 diabetes; BP = blood pressure; SBP = systolic blood pressure; DBP = diastolic blood pressure; HR = heart rate; ID = intraduodenal; MAP = mean arterial pressure; PD = Parkinson's disease; MSA = multi-system atrophy; PPH = postprandial hypotension; OH = orthostatic hypotension; CHO = carbohydrate; GLP-1 = glucagon-like peptide-1; AUC = area under the curve; NS = not significant; M = male; F = female.

Table 5.2. Studies relating to the effects of other nutritive sweeteners on blood pressure.

Study	Year	Participant characteristics	Study Design	Sweeteners assessed rather than glucose	Test meal	Effects on blood pressure
Brown et al. [359]	2008	15 healthy normal-weight volunteers, 9M:6F, aged 24 ± 1 years	Randomised crossover study	Fructose	500 mL of either water, 60g glucose, or 60g fructose.	Oral fructose, but not glucose, significantly increased SBP and DBP. The maximum rise in SBP after fructose was 6.2 ± 0.8 mmHg.
Charriere et al. [370]	2017	9 young healthy men, aged 24 ± 1 years	Randomised crossover study	Galactose, fructose	500 mL of water containing 60g of either glucose, fructose or galactose.	The increase in SBP after fructose (7–8 mmHg) was greater than after glucose (4–5 mmHg) or galactose (2–3 mmHg). DBP increased to a greater extent with fructose (~5 mmHg), compared to non-significant increases of only 2–3 mmHg after glucose or galactose.
Gentilcore et al. [18]	2005	8 healthy older participants, 5M:3F, aged 65-79 years	Randomised crossover study	Sucrose	300 mL drink of 100g sucrose and 30 mL lemon juice with or without 100mg acarbose	There was a fall in SBP and DBP on the control day while there was an overall increase in SBP on the acarbose day.
Gentilcore et al. [107]	2011	8 healthy older participants, 4M:4F, aged 66–77 years	Randomised crossover study	Sucrose	ID infusion of sucrose (100g/300 mL) at ~6 kcal/min with or without acarbose (100mg), over 60min.	There were significant falls in SBP (maximum fall: 11.2 ± 2.0mmHg) during control, but not after acarbose. The fall in DBP was greater after control (10.9 ± 0.9mmHg) than after acarbose (8.1 ± 1.5mmHg).
Grasser et al. [360]	2014	12 healthy young adults, 7M:5F, aged 22.0 ± 0.4 years	Randomised crossover study	Fructose, sucrose	500 mL drink of either 60g sucrose, 60g glucose, 60g fructose or 30g fructose.	Ingestion of fructose (60 or 30g) elevated SBP, DBP and MAP. Ingestion of glucose elevated SBP. Ingestion of sucrose showed no BP changes. The increases in DBP and MAP were significantly higher for fructose (60 or 30 g) than for either glucose or sucrose. The increase in SBP was significantly higher for fructose than for sucrose.
Jansen et al. [134]	1987	10 young normotensive people, aged 28 ± 1 years (YN), 10 young hypertensive patients, aged 44 ± 2 years (YH), 10 elderly	Randomised crossover study	Fructose	300 mL drink of either 75g glucose or 75g fructose	Glucose decreased MAP significantly in the EH, EN and YH group. After fructose, BP remained unchanged in 4 groups.

		normotensive people aged 75 ± 2 years (EN), 10 elderly hypertensive patients aged 75 ± 1 years (EH)				
Mathias et al. [40]	1989	6 patients with chronic autonomic failure (CAF), 4M:2F, aged 42-68 years; 6 age-matched participants without CAF, aged 45-70 years; and 8 normal participants, all M, aged 28-35 years	Randomised parallel study	Xylose	An iso-osmotic solution of glucose (1g/kg body weight) or xylose (0.83g/kg body weight) in 250 mL water.	Xylose caused a lower and more transient fall in BP than glucose in patients with CAF (15 ± 6% vs. 34 ± 7%). After glucose, there was a substantial fall in 6 age-matched participants but a minimal change in 8 male normal participants.
Robinson et al. [42]	1992	5 people with age-related OH, 2M:3F, aged 73-88 years, 3 people with autonomic failure, 1M:2F, aged 72-79 years and 5 controls, 2M:3F, aged 72-86 years	Randomised crossover study	Xylose	50g glucose or 42g xylose in 100 mL water	There were no significant BP changes in the control group. In OH and autonomic failure groups, the SBP decreased comparably following glucose and xylose, DBP was lowered 60-90min after glucose.
Teunissen-Beekman et al. [386]	2014	48 participants, 31M; 17F, aged 58 ± 1 (SEM) years	Randomised crossover study	Maltodextrin, sucrose	Test drink of 70g either protein (pea protein isolate, milk protein isolate, egg white protein isolate or mixed protein), sucrose or maltodextrin.	DBP and MAP were significantly decreased after maltodextrin, but not after protein mix or sucrose. SBP was not significantly changed after any of the meals.
Vanis et al. [104]	2011	8 healthy older participants, 6M:2F, aged 65-75 years	Randomised crossover study	Xylose	300 mL drink of water, 50g glucose or 50g d-xylose.	There was a fall in SBP after glucose drink and no change after xylose or water drink.
Visvanathan et al. [133]	2005	10 healthy older participants, 4M:6F, aged 72.2 ± 1.50 years	Randomised crossover study	Fructose, sucrose	300 mL of either 50g glucose, 50g sucrose, 50g fructose or water + 30 mL lemon juice	SBP decreased significantly following glucose (-3.96 ± 1.38mmHg) and sucrose (-3.03 ± 1.37 mmHg), but not fructose, ingestion (2.59 ± 1.62 mmHg). The decrease in SBP occurred earlier after glucose than sucrose ingestion (7.33 ± 2.19 vs 21.0 ± 4.30 min).

T2D = type 2 diabetes; BP = blood pressure; SBP = systolic blood pressure; DBP = diastolic blood pressure; ID = intraduodenal; MAP = mean arterial pressure; MSA = multi-system atrophy; PPH = postprandial hypotension; OH = orthostatic hypotension.

Table 5.3. Studies relating to the effects of non-nutritive sweeteners on blood pressure.

Study	Year	Participant characteristics	Study Design	Non-nutritive sweeteners assessed	Test meal	Effects on blood pressure
Kazmi et al. [414]	2017	200 students divided equally into 4 groups: A (aged 18.82 ± 0.80 years), B: (aged 18.60 ± 0.57), C (aged 18.64 ± 0.59) and D (18.64 ± 0.59)	Parallel study	Aspartame, Acesulfame-K, sucralose	Group A (control): 10g of cellulose. Group B: 0.36gm (5mg/kg) sucralose. Group C: 10.8g (150mg/kg) aspartame. Group D: 3.24g (45 mg/kg) Acesulfame-K.	There was no difference in BP between group A and B. SBP was lower at 60, 90 and 120min for group C; and at 60 minutes for group D compared to control.
Pham et al. [366]	2018	12 healthy participants, 6M: 6F, aged 73.2 ± 1.1 (SEM) years	Randomised crossover study	Sucralose	ID infusion of either glucose (25%, ~1400 mOsmol/L), sucralose (4 mmol/L, ~300 mOsmol/L) or saline (0.9%, ~300 mOsmol/L) at a rate of 3 mL/min for 60min followed by ID saline for a further 60min.	MAP decreased during glucose but not during sucralose or saline. By t = 60min, MAP was lower after glucose (85.9 ± 2.8 mmHg) than after sucralose (93.1 ± 2.2 mmHg) infusions without significant difference between sucralose and saline infusions.

BP = blood pressure; MAP = mean arterial pressure; ID = intraduodenal.

Chapter 6

Effects of intraduodenal administration of the artificial sweetener, sucralose, on blood pressure and superior mesenteric artery blood flow in healthy older subjects

Statement of Authorship

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Overall percentage	70%
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.

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By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

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6.1 Introduction

Postprandial hypotension (PPH) is now recognised as a frequent and important clinical problem, particularly in the healthy elderly (24 – 38%) [5] and in autonomic dysfunction (e.g. diabetes (~40%) [1, 5] and Parkinson's disease (PD) (40 – 100%)) [1, 5]. It is frequently associated with syncope and falls [1, 4] as well as increased mortality [68].

The pathophysiology of PPH is multifactorial and incompletely understood. It involves the interplay between autonomic and neural mechanisms, including the release of gut hormones, which are influenced by meal composition, gastric distension, and small intestinal nutrient delivery. After a meal, there is a doubling of superior mesenteric artery (SMA) blood flow [1]. In healthy young individuals with intact baroreflex function, the increase in splanchnic blood flow is accompanied by concomitant increases in heart rate (HR), peripheral vascular resistance, stroke volume and cardiac output [12]. In patients with PPH, these compensatory responses are inadequate [1]. We have shown that the postprandial fall in BP is greater when gastric emptying is more rapid [7], whereas, gastric distension attenuates the fall in BP in healthy young and older, people [141-143].

All macronutrients reduce BP comparably [9, 83, 128, 130]; we have shown that the hypotensive responses to fat and protein are similar, but occur slightly later, than the response to glucose [9], probably reflecting the more prolonged time for digestion. The effects of carbohydrate on glycaemia/insulinaemia are not apparently related to the hypotensive response (e.g. *iv* glucose has little effect on BP and PPH occurs frequently in type 1 diabetes (T1D) [5]. Studies by our group [413, 430] and others [417, 431] have highlighted the importance of sweet taste receptors (STRs; T1R2/T1R3) in the proximal intestine in the detection of luminal glucose and artificial sweeteners, and release of gut hormones, particularly the 'incretin hormones',

glucose-dependent insulinotropic peptide (GIP) [413] and glucagon-like peptide-1 (GLP-1) [413, 415, 417, 432]. Intestinal STRs also specifically regulate levels of the intestinal glucose transporter, sodium-glucose cotransporter-1 (SGLT-1). We have shown that xylose, a poorly absorbed pentose sugar, empties comparably to glucose, but has no effect on BP in healthy older subjects [104], as is the case for fructose [133]. While sugars and artificial sweeteners are perceived by lingual taste as equivalent, neither xylose nor fructose is a substrate for SGLT-1, suggesting a role for SGLT-1 in mediating the hypotensive response to carbohydrate, rather than sweet taste per se. Non-caloric artificial sweeteners, including sucralose, are now used widely as a substitute for carbohydrate. Surprisingly, there is no information about the effect of artificial sweeteners on postprandial BP.

We aimed to evaluate the effects of intraduodenal (ID) administration of the artificial sweetener, sucralose, compared to glucose and saline, on BP and SMA blood flow in healthy older subjects.

6.2 Materials and methods

6.2.1 Subjects

The study included twelve healthy, volunteers (six male, and six female, mean age 73.2 ± 1.1 years, BMI 26.9 ± 0·9 kg/m^2). All subjects were non-smokers, and none had a history of gastrointestinal disease or surgery, diabetes, significant respiratory, renal or cardiac disease, chronic alcohol abuse, epilepsy, or were taking medication known to influence BP or gastrointestinal function.

The study was conducted in accordance with the Declaration of Helsinki, and all procedures involving human subjects were approved by the Human Research Ethics Committee of the Royal Adelaide Hospital. Written informed consent was obtained from all subjects.

6.2.2 Protocol

Each subject was studied on 3 occasions, separated by at least 7 days, in a randomised, double-blind, crossover design. Randomisation was performed using a computer-generated program. On each study day, the subject attended the Discipline of Medicine, Royal Adelaide Hospital, the University of Adelaide, at ~ 09.00 h after a fast (14 h for solids; 12 h for liquids) [75]. A multi-lumen silicone catheter (~ 3.4-mm diameter) (Dentsleeve International Ltd, Mui Scientific, Mississauga, Canada), which included an infusion channel with a port located ~12 cm distal to the pylorus (i.e. in the duodenum) and 2 other channels, 1 positioned in the antrum (2.5 cm proximal to the pylorus) and the other in the duodenum (2.5 cm distal to the pylorus), was introduced into the stomach via an anesthetised nostril [75]. The latter 2 channels were perfused with normal saline (0.9%). The correct positioning of the catheter was maintained by continuous measurement of the transmucosal potential difference between the antral (−40 mV) and the duodenal (0 mV) channels [228]. For this purpose, an intravenous cannula filled with sterile saline was placed subcutaneously in the left forearm and used as a reference electrode [228]. The tip of the catheter was allowed to pass into the duodenum by peristalsis, which took between t = 30 and 180 min. When the catheter was positioned correctly, the subject was placed in the supine position. An intravenous cannula was inserted into an antecubital vein for blood sampling and an automated BP cuff placed around the opposite arm.

Following a stabilisation period of at least 15 min, subjects received an ID infusion of glucose (25%w/v ~1400mOsmol/L), sucralose (Tate & Lyle, Decatur, IL, USA) (4mmol/L, ~300mOsmol/L) or saline (0.9%w/v ~300mOsmol/L) at a rate of 3mL/min (i.e. 3 kcal/min for the glucose solution) from t = 0 – 60 min. ID infusions were administered via a volumetric infusion pump (Gemini PC-1; IMED Corp, San Diego, CA, USA) and both the subject and the study investigators were blinded to the study condition. On all study days, saline (0.9%w/v) was infused at a rate of 3mL/min between t = 60 and 120 min. All solutions were infused at room temperature (~22°C). At t = 120 min, the catheter was removed, subjects offered a light lunch and a final blood sample and BP measurement were taken. Subjects were permitted to leave the laboratory once their BP and blood glucose had returned to baseline levels.

6.2.3 Measurements

6.2.3.1 Blood pressure and heart rate

Systolic and diastolic BP (SBP and DBP) and HR were measured with an automated oscillometric BP monitor (DINAMAP ProCare 100, GE Medical Systems, Milwaukee, WI, USA) at t = -9, -6, and -3 min before commencement of the ID infusion and, subsequently, every 3 min between t = 0 and 120 min. Baseline BP and HR were calculated as the mean of measurements obtained at t = -9, -6, and -3 min before commencement of the ID infusion. The mean arterial pressure (MAP) was calculated using the formula MAP = DBP + [(SBP - DBP)/3].

6.2.3.2 Superior mesenteric artery blood flow

SMA blood flow was measured at t = -2 min, and then every 15 min between t = 0 – 120 min using a Logiq e™ ultrasound system with a 3.5 C broad-spectrum 2.5 – 4 MHz convex linear

array transducer (GE Healthcare Technologies, Sydney, NSW, Australia). SMA blood flow (mL/min) was calculated instantaneously by using the following equation [433]:

$$\text{Blood flow (mL/min)} = \pi \times r^2 \times \text{TAMV} \times 60$$

where r = the radius of the SMA, and TAMV is the time-averaged mean velocity.

6.2.3.3 Blood glucose concentrations

Venous blood samples (~ 15 mL) were obtained immediately prior to commencement of the infusion (t = −2 minutes) and at t = 15, 30, 45, 60, 90 and 120 min. Blood glucose concentrations were determined immediately using a portable blood glucose meter (Medisense Companion 2 Meter, Medisense Inc., Waltham, MA, USA) [434, 435].

6.2.4 Statistical analysis

MAP, HR, SMA blood flow and blood glucose concentrations were analysed and presented as absolute values. One-way ANOVA was used to evaluate the effects of time on absolute values for MAP, HR, SMA blood flow and blood glucose. The maximum falls in MAP and rise in HR, SMA blood flow, blood glucose concentrations were defined as the greatest change from baseline in each subject at any given time point for each treatment. Areas under the curve (AUCs) were calculated using the trapezoidal rule and analysed by one-way ANOVA to evaluate a treatment effect from t = 0 to 60 min and from t = 0 to 120 min for MAP and HR, from t = −2 to 60 min and from t = -2 to 120 min for SMA blood flow, and blood glucose. All analyses were performed using SPSS version 23 (SPSS, Chicago, IL, USA). Data are presented as mean values ± SEM, unless stated otherwise. A P value < 0.05 was considered significant in all analyses.

6.3 Results

The studies were well tolerated. One subject experienced symptoms referable to hypoglycaemia (dizziness, headache, sweating, extreme hunger and thirst; blood glucose 2.8mmol/L at only 1 time point i.e. t = 120 min). The symptoms resolved promptly following ingestion of a glucose drink and all data were included in the analysis.

6.3.1 Blood pressure and heart rate

There were no differences in baseline (t = 0 min) BP or HR among the 3 days (saline vs. sucralose vs. glucose, respectively) (Table 6.1).

6.3.1.1 Mean arterial pressure

Between t = 0 and 60 min, there was a fall (P = 0.001) in MAP with glucose, but no overall change during either sucralose (P = 0.32) or saline (P = 0.59), infusions (Figure 6.1A). There was a treatment effect (P = 0.005) for the AUC of the change in MAP, so that MAP was lower during glucose (P = 0.035) compared with saline, without any difference between sucralose and saline (P = 1.0) or sucralose and glucose (P = 0.071). At the end of the glucose infusion at t = 60 min, MAP (85.9 ± 2.8 mmHg) was lower than saline (95.3 ± 2.5 mmHg, P = 0.001) and sucralose (93.1 ± 2.2 mmHg, P = 0.01) infusions, while there was no significant difference in MAP between sucralose and saline infusions (P = 0.54) (Figure 6.1A).

Between t = 0 and 120 min, there was a treatment effect (P = 0.001) for the AUC of the change in MAP, so that MAP was lower on the day with glucose, when compared with both saline and sucralose, infusions (P = 0.021 for both), while there was no difference between saline and sucralose infusions (P = 1.0) (Figure 6.1A). The maximum fall in MAP from baseline was

greater (P = 0.019) during glucose (11.6 ± 2.0 mmHg), compared with saline, infusion (5.1 ± 0.6 mmHg).

At t = 120 min, MAP did not differ from baseline after glucose infusion (93.3 ± 3.9 mmHg, P = 0.62), but was greater after sucralose (98.3 ± 2.9 mmHg, P = 0.043) and saline (100 ± 3.0 mmHg, P < 0.001) infusions.

6.3.1.2 Heart rate

Between t = 0 and 60 min, there was a rise in HR during the glucose (P < 0.0001), but no change during the sucralose (P = 0.12) or saline (P = 0.12), infusions (Figure 6.1B). While there was an overall treatment effect (P = 0.034) for the AUC of the change in HR, there were no significant differences between the three infusions - i.e., glucose vs. sucralose (P = 0.09), glucose vs. saline (P = 0.26) and sucralose vs. saline (P = 1.0).

Between t = 0 and 120 min, there was a treatment effect (P = 0.008) for the AUC of the change in HR, so that HR was greater on the day with glucose infusion, when compared with sucralose (P = 0.047) infusions, while there was no difference between saline infusion and sucralose (P = 1.0) or glucose infusions (P = 0.07) (Figure 6.1B). The maximum rise in HR from baseline was greater (P = 0.007) on the study with glucose (15 ± 2 bpm) compared with saline (6.5 ± 1.2 bpm) infusion. There was no significant difference in the maximum rise in HR between the sucralose (8.8 ± 0.98 bpm), saline (P = 0.37) and glucose (P = 0.09) infusion study days.

At t = 120 min, HR was greater than baseline after the three infusions (saline vs. sucralose vs. glucose: 63 ± 2 bpm, P = 0.01 vs. 62 ± 2 bpm, P < 0.001 vs. 67 ± 3 bpm, P < 0.01, respectively).

6.3.2 Superior mesenteric artery blood flow

There was no difference (P = 0.85) in baseline (t = −2 min) SMA blood flow among the 3 days (Table 6.1).

Between t = -2 and 60 min, there was a rise in SMA blood flow with the glucose (P < 0.0001), but no significant change with the sucralose (P = 0.15) or saline (P = 0.22), infusions (Figure 6.2).

Between t = -2 and 60 min or t = -2 and 120 min, there was a treatment effect (P < 0.001) for the AUC of SMA blood flow, so that SMA blood flow was greater during glucose, compared with sucralose and saline (P < 0.001 for both) infusions without any difference between sucralose and saline (P = 1.0) (Figure 6.2). The maximum rise in SMA blood flow during glucose infusion (514 ± 61 mL/min) was greater than during saline (53 ± 14 mL/min) and sucralose (43 ± 14mL/min) infusions (P < 0.001 for both), while there was no difference in the maximum rise in SMA blood flow between sucralose and saline infusions (P = 1.0).

At t = 120 min, SMA blood flow did not differ from baseline among the 3 days (saline vs. sucralose vs. glucose: 281 ± 12 mL/min, P = 0.28 vs. 300 ± 18 mL/min, P = 0.75 vs. 308 ± 26 mL/min, P = 0.98, respectively) (Figure 6.2).

6.3.3 Blood glucose

There was no difference (P = 0.43) in baseline (t = −2 min) blood glucose among the 3 days (Table 6.1).

Between t = -2 and 60 min, there was a rise in blood glucose during the glucose ($P < 0.0001$) but no change during the sucralose ($P = 0.66$) or saline ($P = 0.77$) infusions (Figure 6.3).

Between t = -2 and 60 min and t = -2 and 120 min, there was a treatment effect ($P < 0.001$) for the AUC of blood glucose, so that blood glucose was higher during glucose, compared with sucralose and saline, infusions ($P < 0.001$ for both) without any difference between sucralose and saline infusions ($P = 0.26$ between t = -2 and 60 min, $P = 0.12$ between t = -2 and 120 min) (Figure 6.3). Peak blood glucose during glucose infusion (11.3 ± 0.43 mmol/L) was also greater than saline (5.3 ± 0.09 mmol/L) and sucralose (5.4 ± 0.07 mmol/L) infusions ($P < 0.001$ for both), while there was no significant difference in the peak blood glucose between sucralose and saline infusions ($P = 1.0$) (Figure 6.3).

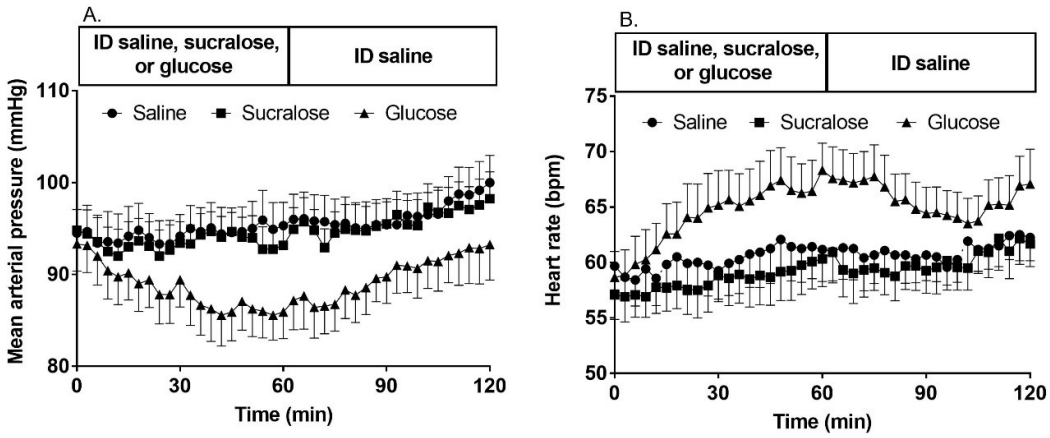

Figure 6.1. Mean arterial blood pressure (A), and heart rate (B) in 12 healthy older subjects following intraduodenal infusion of glucose, sucralose, and saline. Infusions were given between t = 0 - 60 min followed by saline. Data are analysed with one-way ANOVA with Bonferroni correction and presented as mean values ± SEM. Mean arterial blood pressure treatment effect between t = 0 and 120 min: $P < 0.05$ for glucose compared with sucralose; $P < 0.05$ for glucose compared with saline; $P = 1.0$ for sucralose compared with saline. Heart rate treatment effect between t = 0 and 120 min: $P < 0.05$ for glucose compared with sucralose; $P = 0.07$ for glucose compared with saline; $P = 1.0$ for sucralose compared with saline.

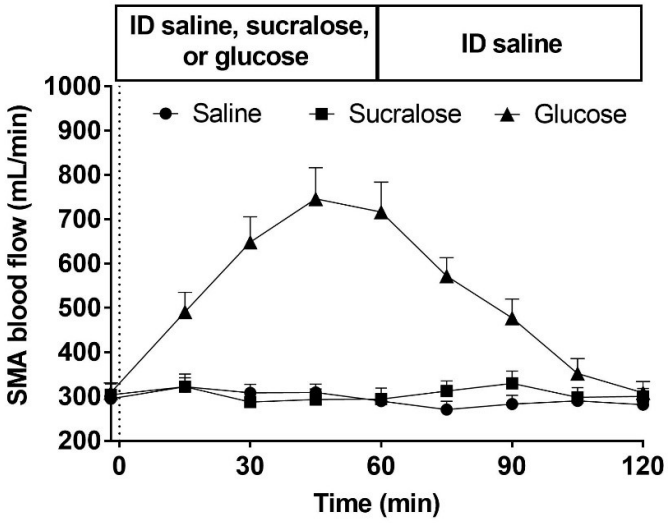

Figure 6.2. Superior mesenteric artery (SMA) blood flow in 12 healthy older subjects following intraduodenal infusion of glucose, sucralose, and saline. Infusions were given between t = 0 - 60 min followed by saline. Data are analysed with one-way ANOVA with Bonferroni correction and presented as mean values ± SEM. Between t = 0 and 120 min: $P < 0.001$ for glucose compared with sucralose; $P < 0.001$ for glucose compared with saline; $P = 1.0$ for sucralose compared with saline.

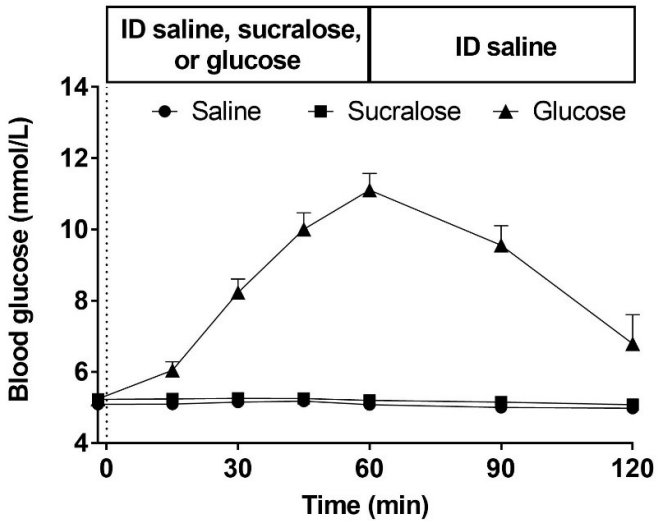

Figure 6.3. Blood glucose concentration in 12 healthy older subjects following intraduodenal infusion of glucose, sucralose, and saline. Infusions were given between t = 0 - 60 min followed by saline. Data are analysed with one-way ANOVA with Bonferroni correction and presented as mean values ± SEM. Between t = 0 and 120 min: $P < 0.001$ for glucose compared with sucralose; $P < 0.001$ for glucose compared with saline; $P = 0.12$ for sucralose compared with saline.

6.4 Discussion

Our study confirms that in healthy older subjects, ID infusion of glucose at a rate of 3 kcal/min is associated with a substantial fall in BP and rises in both HR and splanchnic blood flow [83]. In contrast, ID infusion of sucralose had no effect on BP, HR or splanchnic blood flow. These outcomes indicate that sucralose does not affect BP and provide a rationale for the use of sucralose, and potentially other artificial sweeteners, in the management of PPH.

In healthy older subjects and patients with type 2 diabetes (T2D), the magnitude of the postprandial fall in BP is greater when gastric emptying is relatively more rapid. By contrast, gastric distension by ingestion of water [15] or a balloon [10, 89] attenuates the fall in BP in response to both oral and ID glucose and consumption of water before a meal has been advocated in the management of PPH [11, 140-142]. In this proof of principle study, we accordingly infused glucose intraduodenally and at a rate with the normal range for gastric emptying (i.e. 3 kcal/min) [220].

A variety of non-caloric artificial sweeteners, including sucralose, are increasingly being used in the food industry as an alternative to sugar, particularly directed to obese people and those with diabetes, with the aim of reducing energy intake and minimizing the carbohydrate load [341]. The dose of sucralose evaluated equates to 4 mmol/L (i.e. 0.067 mmol/L/min), which is a substantially greater osmotic load than that usually ingested in the diet.

A simple carbohydrate meal has been reported to have a greater hypotensive effect than an isocaloric complex carbohydrate meal in healthy older people, which is likely to reflect different rates of absorption in the small intestine [132]. In healthy older subjects, while both glucose and sucrose lead to comparable reductions in BP, fructose [133, 134] and xylose [40,

103] have no effect on BP. These observations suggest that the hypotensive effect of glucose is probably not mediated by STRs per se, but rather by another glucose-specific factor [134], such as SGLT-1, that has the capacity for substantial modulation of GLP-1 or GIP secretion.

Several studies have confirmed the presence of heterodimeric sweet taste receptors (STRs, T1R2+TR3) in intestinal brush and enteroendocrine cells at the human upper gastrointestinal tract, which are analogous to lingual sweet taste receptors [430, 436-438]. They are paired with the G-protein, α-gustducin, to activate the transient receptor potential cation channel M5 (TRPM5) [439]. STRs play an important role in perceiving sugars and artificial sweeteners in intestinal lumen as well as in stimulating the secretion of gut hormones, especially incretin hormones such as GLP-1 and GIP [439-441].

The postprandial increase in splanchnic blood flow is likely to be integral to the fall in BP. Octreotide, which is known to suppress the release of gut hormones, attenuates both the postprandial increase in SMA blood flow, and the postprandial fall in BP in patients with autonomic failure [137]. Our observation that ID sucralose did not affect SMA blood flow, is not surprising given the absence of an effect on BP and may be because sucralose has no effect on insulin, GLP-1 or GIP release [413, 415]. A comparable mesenteric hyperaemia following the glucose drink is observed in both healthy subjects and patients with autonomic failure, but only patients with autonomic failure experience falls in BP, suggesting that PPH appears to result from inadequate sympathetic compensation [78]. Moreover, glucose consumption results in an increase in the sympathetic nervous system activity [442], which aggravates the situation. Meredith et al. found that non-caloric sugars did not stimulate sympathetic activity in the rat [443], however, there has been no information about the effect of artificial sweeteners specifically on human sympathetic nervous system activity.

Limitations of our study should be acknowledged. It was not feasible to match sucralose and glucose solutions for osmolarity, and while we cannot exclude a potential effect, we have reported that glucose concentration does not affect the postprandial hypotensive response to ID glucose in healthy older subjects [75] and that neither xylose [104] nor fructose [133] had an effect on BP. We did not assess whether sucralose could modify the response to carbohydrate, such as glucose, however, both an acute [432] and a 12 week-daily sucralose [431] consumption had no effect on the glucose absorption. This 'proof of principle' study had a relatively small sample size and insulin, GLP-1 and GIP were not assessed. Finally, observations relating to sucralose should not be extrapolated to other non-caloric sweeteners.

In conclusion, this study has demonstrated that acute ID administration of sucralose does not influence postprandial BP, HR and splanchnic blood in healthy human subjects. Accordingly, although further work in this area is needed, artificial sweeteners may have therapeutic benefit in the dietary management of PPH.

Table 6.1. Baseline characteristics of the healthy older subjects (n = 12)[1]

	Saline	Sucralose	Glucose	P-value
SBP (mmHg)	138 ± 6	140 ± 5	134 ± 5	0.12
DBP (mmHg)	71 ± 2	71 ± 2	70 ± 2	0.33
MAP (mmHg)	93.5 ± 2.9	94.5 ± 2.4	91.7 ± 2.6	0.075
HR (beats/min)	59 ± 2	57 ± 2	58 ± 2	0.44
SMA blood flow (mL/min)	295 ± 13	304 ± 24	309 ± 22	0.85
Blood glucose (mmol/L)	5.1 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	0.43

[1] All values are absolute values. Differences between study days were tested via one-way ANOVA analyses. Values are means ± SEMs. SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; SMA, superior mesenteric artery.

Chapter 7

A randomised, crossover study of the acute effects of acarbose and gastric distension, alone and combined, on postprandial blood pressure in healthy older adults

Statement of Authorship

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Name of Principal Author (Candidate)	Hung T Pham
Contribution to the Paper	Conducted research, analysed and interpreted data, wrote and revised paper.
Overall percentage	70%
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements

	with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	Aug 2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

Name of Co-Author	Laurence G Trahair		
Contribution to the Paper	Conducted research, analysed data and reviewed paper.		
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Name of Co-Author	Michael Horowitz		
Contribution to the Paper	Conceived and designed research, interpreted data and reviewed paper.		
Signature		Date	Aug 2019

Name of Co-Author	Karen L Jones		
Contribution to the Paper	Conceived and designed research, interpreted data, reviewed paper and approved final version of paper.		
Signature		Date	Aug 2019

7.1 Introduction

Postprandial hypotension (PPH), usually defined as a fall in systolic blood pressure (BP) of ≥ 20 mmHg, within 2 hours of a meal [1, 4], occurs frequently, e.g. in 24–38% of 'healthy' older adults [68] and 25-40% of patients with type 2 diabetes (T2D) [1, 5], and is associated with major adverse sequelae, including syncope and falls [1, 4], as well as increased mortality [68]. Current management is suboptimal [5].

The pathophysiology of PPH is heterogeneous, which may account for the lack of effective management. Multiple factors appear to be involved, including autonomic dysfunction, the release of gastrointestinal hormones, meal composition, gastric distension, and small intestinal nutrient delivery. After a meal, there is substantial splanchnic blood pooling [1], and in healthy young individuals, baroreflex mechanisms protect against a post-meal decrease in BP through compensatory increases in heart rate (HR), stroke volume and cardiac output [12]. In 'healthy' older adults and in patients with PPH, these compensatory responses are inadequate to maintain BP [1]. We have shown in healthy older adults and people with T2D that the magnitude of the postprandial fall in BP is greater when the rate of gastric emptying [7], or direct small intestinal nutrient delivery [8, 24], is relatively more rapid. For example, when glucose is infused into the duodenum at rates spanning the normal physiological range, a 3 kcal/min load induces a much greater decrease in BP and rises in HR and superior mesenteric artery (SMA) blood flow than 1kcal/min [8]. Moreover, PPH is associated with more rapid gastric emptying [74]. In contrast to the effect of small intestinal nutrient delivery, gastric distension appears to be protective in PPH. Drinking water with a meal has been reported to attenuate the postprandial fall in BP in healthy older adults [141, 142], as well as in patients with autonomic failure [141-143], probably primarily by inducing gastric distension. For example, Gentilcore et al. (2008) reported that gastric distension with water at a volume as low as 300 mL diminished the fall in

BP in response to intraduodenal (ID) glucose in the healthy elderly [15]. The pressor response to water may also be greater in patients with autonomic failure [142].

The α-glucosidase inhibitor, acarbose, is used widely in the management of T2D. By delaying intestinal disaccharide absorption, it reduces postprandial glycaemia [164]. Acarbose also stimulates the release of the incretin hormone, glucagon-like peptide-1 (GLP-1) [168], and slows gastric emptying [162], which may contribute to glucose-lowering in T2D. We reported previously that acarbose (100mg) attenuated the fall in systolic BP induced by oral sucrose in healthy older adults, an effect associated temporally with slowing of gastric emptying and stimulation of GLP-1 [18]. However, when administered intraduodenally i.e. bypassing any effect of slowing gastric emptying, acarbose also attenuated the fall in BP induced by sucrose [107], which may reflect an effect of acarbose to reduce splanchnic blood flow [107]. Subsequent studies support the efficacy of acarbose in the management of PPH to attenuate [167, 169], but not abolish, the fall in BP, although these have, for the main part, employed small cohorts and associated with substantial methodological limitations. Somewhat surprisingly, only one study has hitherto evaluated the effect of acarbose (50mg) on the splanchnic blood flow response to a meal [165], which appears central to the hypotensive response. Furthermore, this study was in a heterogeneous group of individuals with PPH and was neither randomised nor blinded in design, compromising meaningful interpretation [165].

While it is apparent that gastric distension (induced by water drinking) and acarbose theoretically have complementary effects to attenuate the postprandial fall in BP, this has hitherto not been evaluated, which is surprising given the simplicity and safety of both approaches. The aim of this study was to determine the acute effects of water drinking and acarbose, alone and combined, on the BP and SMA blood flow responses to oral sucrose. To

establish 'proof-of-principle', we studied a cohort of healthy older adults, rather than patients with PPH.

7.2 Materials and methods

7.2.1 Subjects

Ten healthy older adults living in the community (2 male, and 8 female, mean age 74.0 ± 1.4 years, BMI 26.2 ± 1.1 kg/m^2) were recruited by advertisements placed around the hospital, in newspapers and via social media, and from a database of subjects who had participated in research studies and consented to be contacted. All healthy subjects were non-smokers and none had a history of gastrointestinal disease or surgery, diabetes, significant respiratory, renal or cardiac disease, chronic alcohol abuse, epilepsy, or was taking medication known to influence BP or gastrointestinal function. The study was conducted in accordance with the Declaration of Helsinki following the provision of written informed consent by each of the participants. The study was registered at http://www.ANZCTR.org.au (ACTRN12618000152224) and approved by the Human Research Ethics Committee of the Royal Adelaide Hospital. The study adheres to CONSORT guidelines.

7.2.2 Protocol

Each subject was studied on 4 occasions, separated by at least 1 week, in a randomised, crossover design. On each study day, the subject attended the University of Adelaide, Discipline of Medicine at the Royal Adelaide Hospital at ~ 09.00 h after an overnight fast (14h for solids; 12h for liquids) [75]. Subjects were seated in a chair, an intravenous cannula was inserted into an antecubital vein for blood sampling and an automated BP cuff placed around the opposite arm. Following a period of 'rest' of 15-30 minutes to allow baseline BP to stabilise

[24], subjects were given, in random order, either the (i) control treatment (C): a test drink comprising 100g sucrose dissolved in 300 mL of water (~407 kcal), (ii) distension treatment (D): a 'preload' of 300 mL water 15 minutes before the ingestion of the test drink, (iii) acarbose treatment (A): 100mg acarbose (Glucobay™, Bayer Schering Pharma AG, Berlin, Germany) dissolved in the test drink or (iv) combined treatment (AD): the water preload was given 15 minutes before the ingestion of acarbose which was dissolved in the test drink. Both the 'preload' and test drink were consumed within 2 minutes with time zero (t = 0 min) defined as the end of test drink ingestion (Figure 7.1).

Measurements of BP, HR and SMA blood flow were performed at regular intervals until t = 120 min, as the greatest fall in postprandial BP is known to occur within that time [68]. At the end of each study day, each subject was provided with lunch and a final BP measurement was taken prior to them leaving the laboratory.

7.2.3 Measurements

7.2.3.1 Blood pressure and heart rate

Systolic and diastolic BP (SBP and DBP) and HR were measured with an automated oscillometric BP monitor (DINAMAP ProCare 100, GE Medical Systems, Milwaukee, WI, USA*)* at *3-min intervals* prior to ingesting the test drink, and, subsequently, every 3 min between t = 0-120 min [7, 8, 15, 18, 22, 23, 75, 140, 155, 197, 444]. An average of BP and HR measurements obtained at t = -24, -21, -18 min prior to the ingestion of the preload (t = -15 min), or, on the study day without a preload, at t = -9, -6, -3 min prior to ingestion of the test drink, were calculated to represent baseline BP and HR. The mean arterial pressure (MAP) was

calculated using the formula MAP = DBP + [(SBP - DBP)/3]. PPH was defined as a fall in systolic BP of at least 20 mmHg that was sustained for 30 min or more [7].

7.2.3.2 Superior mesenteric artery blood flow

SMA blood flow (mL/min) was measured prior to the ingestion of the preload (t = -17 min) and/or test drink (t = −2 min), and then every 15 min between t = 0-120 min using a Logiq e™ ultrasound system (GE Healthcare Technologies, Sydney, NSW, Australia) incorporating a 3.5C broad-spectrum 2.5–4 MHz convex linear array transducer [433].

7.2.3.3 Blood glucose concentrations

Venous blood samples (~15 mL) were drawn immediately before ingestion of the preload (t = -17 min) and /or test drink (t = -2 min) and then at t = 30, 60, 90 and 120 min. Blood glucose concentrations were determined during the study using a portable glucometer (Medisense Companion 2 Meter, Medisense Inc., Waltham, MA, USA) [434].

7.2.3.4 Autonomic nerve function

Autonomic nerve function was assessed on one of the four days using standardised cardiovascular reflex tests with an appropriately sized BP cuff in a temperature-controlled, quiet, clinical study room (ANX-3.0 autonomic monitoring system (ANSAR Medical Technologies, Inc., Philadelphia, PA)) [445-447]. Testing was performed in the fasting state, preceded by 10-15 minutes of rest in the recumbent position. Parasympathetic function was evaluated by: i) the variation (R-R interval) of the HR during deep breathing whereby 6 respiratory cycles were performed and E/I ratio calculated from the peak expiratory and peak inspiratory heartbeat intervals and ii) the response to standing (30:15 ratio), with the relevant

ECG parameters captured and analysed electronically [445]. Sympathetic function was assessed by the fall in systolic BP in response to standing whereby baseline BP was taken following 10-15 minutes in a semi-recumbent position immediately and 3 minutes after standing. An abnormal BP response to standing was defined as a decrease in BP of 20/10mmHg or more upon standing over resting. Each of the test results was scored according to predefined criteria that were adjusted for age where 0 was considered normal, 1 as borderline, and 2 as abnormal providing a total maximum score of 6 [447]. A score of at least 3 was considered to suggest autonomic dysfunction.

7.2.4 Statistical analysis

The maximum fall in MAP and rise in HR were defined as the greatest change from baseline in each subject at any given time point for each treatment. Areas under the curve (AUCs) were calculated using the trapezoidal rule from t = 0-120 min for MAP and HR, and from t = -2-120 min for SMA blood flow and blood glucose. Repeated measures two-factor ANOVA was used to evaluate the effects of acarbose, gastric distension and their interaction for the AUCs for MAP, HR, SMA blood flow and blood glucose. All analyses were performed using SPSS version 23 (SPSS, Chicago, IL, USA). MAP and HR are presented as change from baseline values and SMA blood flow and blood glucose concentrations as absolute values. Data are presented as mean values ± SEM, unless stated otherwise. A P value <0.05 was considered significant in all analyses.

7.3 Results

The studies were reasonably well tolerated. Flatulence and/or diarrhoea were reported by 3 of the 10 subjects on acarbose days; in each case, the onset of symptoms was about 3-4 hours after

ingestion. Three subjects fainted during the study (2 subjects on the distension day and 1 on the acarbose day between t = 60-75 min), which, in each case, resolved promptly after lying supine for 15-30 min. In 1 of the 3 subjects, fainting was concordant with PPH on the distension day. No subject had autonomic neuropathy (mean score: 1.4 ± 0.2). In 1 subject, the ultrasound images of SMA blood flow were suboptimal on all study days due to the presence of bowel gas, precluding their use. In another subject, insertion of the intravenous cannula for blood sampling was unsuccessful on all 4 study days due to poor venous access. Accordingly, BP and HR data are available in 10, while SMA blood flow and blood glucose data are available in 9, subjects.

7.3.1 Blood pressure and heart rate

There were no differences in baseline BP or HR among the 4 treatments (control vs. distension vs. acarbose vs. combined treatment, respectively): SBP (133 ± 3.7 mmHg vs. 128 ± 2.8 mmHg vs. 125 ± 3.6 mmHg vs. 129 ± 3.7 mmHg; P = 0.19); DBP (72 ± 2.6 mmHg vs. 73 ± 2.8 mmHg vs. 70 ± 1.9 mmHg vs. 73 ± 1.7 mmHg; P = 0.23); MAP (92 ± 2.2 mmHg vs. 91 ± 2.4 mmHg vs. 88 ± 2.1 mmHg vs. 92 ± 1.9 mmHg; P = 0.18) and HR (67 ± 2.1 beats/min vs. 68 ± 2.5 beats/min vs. 66 ± 2.8 beats/min vs. 67 ± 2.6 beats/min; P = 0.76). One subject on the control day and another on the distension day had PPH. No subject experienced PPH on either of the acarbose study days.

7.3.1.1 Mean arterial pressure

Between t = 0-120 min, there was a fall in MAP during control and distension treatments (P < 0.001 for both), but no overall change during either acarbose (P = 0.44), albeit a non-significant trend for combined (P = 0.06), treatments. There was a treatment effect for acarbose, so that the $AUC_{0\text{-}120min}$ for MAP was greater during treatments with acarbose (A: 10,625 ± 237

mmHg.min and AD: 10,721 ± 232 mmHg.min; P = 0.005) compared to control (C: 10,366 ± 281 mmHg.min), but not for distension (D: 10,106 ± 252 mmHg.min; P = 0.68). There was also no interactive effect between acarbose and distension on the $AUC_{0\text{-}120min}$ for MAP (P = 0.44) (Figure 7.2A).

The maximum fall in MAP from baseline was less during treatments with acarbose (P = 0.006) (acarbose: -6.9 ± 1.8 mmHg and combined treatment: -8.2 ± 1.5 mmHg) compared with control (-12.2 ± 1.4 mmHg). There was no effect of gastric distension alone (-15.3 ± 1.8 mmHg, P = 0.21) and no difference between the acarbose treatments with or without gastric distension (P = 0.58).

7.3.1.2 Heart rate

Between t = 0-120 min, there was a rise in HR among 4 treatments (P < 0.005 for all) (Figure 7.2B). There was a treatment effect for acarbose, so that the $AUC_{0\text{-}120min}$ for HR was lower during treatments with acarbose (A: 8057 ± 356 bpm.min and AD: 7985 ± 315 bpm.min; P = 0.04) compared to control (C: 8252 ± 357 bpm.min), but not for distension (D: 8165 ± 312 bpm.min; P = 0.55). There was also no interaction between acarbose and distension on the $AUC_{0\text{-}120min}$ for HR (P = 0.92) (Figure 7.2B).

There was no treatment effect for the maximum rise in HR from baseline among the 4 treatments, so that there was no significant difference in the maximum rise in HR during acarbose (10.4 ± 1.4 bpm, P = 0.93), distension (10.5 ± 1.9 bpm, P = 1.0) and combined treatments (10.6 ± 2.4 bpm, P = 0.88), compared to control (10.7 ± 2.5 bpm).

7.3.2 Superior mesenteric artery blood flow

There was no difference (P = 0.83) in baseline (t = -2 min) SMA blood flow among the 4 treatments (control vs. distension vs. acarbose vs. combined treatment: 543 ± 55 mL/min vs. 583 ± 91 mL/min vs. 538 ± 75 mL/min vs. 579 ± 50 mL/min, respectively) (Figure 7.3).

Between t = -2-120 min, there was a rise in SMA blood flow with the control (P < 0.001) and distension (P = 0.02), and a trend for an increase after acarbose alone (P = 0.08) and combined (P = 0.07), treatments (Figure 7.3). There was a treatment effect for the $AUC_{0\text{-}120min}$ of SMA blood flow for acarbose, so that SMA blood flow was less during acarbose treatments, with or without distension (A: 86,689 ± 10,725 mL/min.min and AD: 87,720 ± 6750 mL/min.min; P = 0.003), compared with control (C: 111,738 ± 12,631 mL/min.min). There was no difference between distension (D: 95,846 ± 11,418 mL/min.min) and control (P = 0.41), and no additive effect between acarbose and distension in the combined treatment (P = 0.15) (Figure 7.3).

The maximum rise in SMA blood flow during acarbose treatments (acarbose: 963 ± 123 mL/min and combined treatment: 983 ± 102 mL/min) was less (P = 0.03), compared to control (1173 ± 111 mL/min). There was no difference between distension (1073 ± 140 mL/min) (P = 0.67) and control and no interaction between acarbose and distension (P = 0.42) in the maximum rise in SMA blood flow (Figure 7.3).

7.3.3 Blood glucose

There was no difference (P = 0.56) in baseline (t = -2 min) blood glucose among the 4 treatments (control vs. distension vs. acarbose vs. combined treatment: 5.6 ± 0.16 mmol/L vs. 5.6 ± 0.14 mmol/L vs. 5.6 ± 0.10 mmol/L vs. 5.7 ± 0.20 mmol/L, respectively) (Figure 7.4).

Between t = -2-120 min, there was a rise in blood glucose with all treatments ($P < 0.001$ for all) (Figure 7.4). There was a treatment effect for the $AUC_{0\text{-}120min}$ for blood glucose for acarbose, so that blood glucose was less during acarbose treatments with and without distension (A: 827 ± 28.9 mmol/L.min and AD: 863 ± 30.3mmol/L.min; $P = 0.03$), compared with control (C: 919 ± 41.9 mmol/L.min). There was no difference between distension (D: 948 ± 53.9 mmol/L.min) and control ($P = 0.12$), nor any additive effect between acarbose and distension when combined ($P = 0.92$) (Figure 7.4).

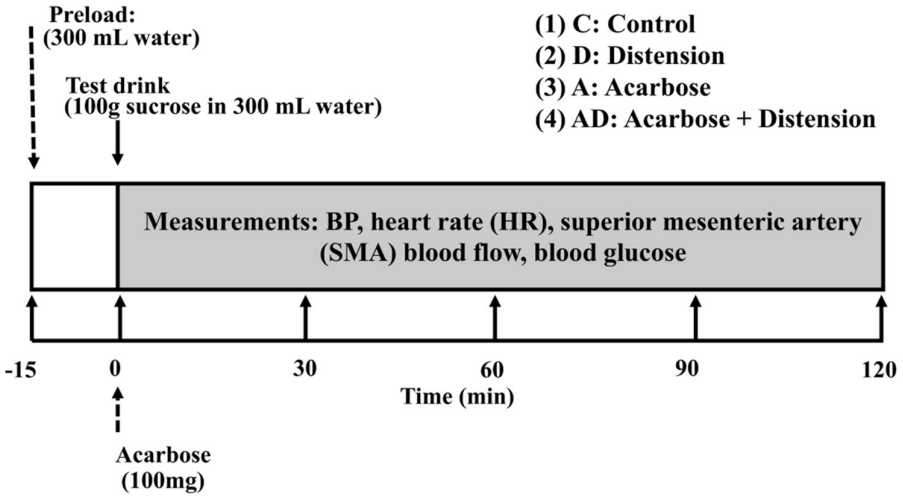

Figure 7.1. Schema of the study protocol.

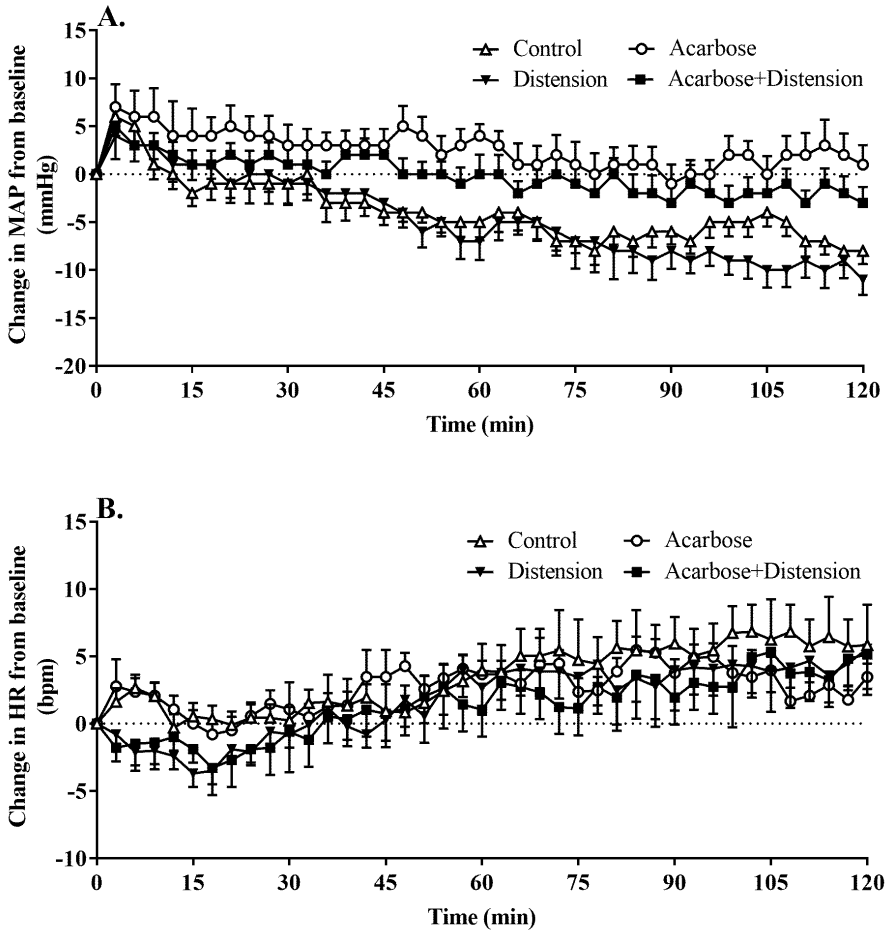

Figure 7.2. Changes in mean arterial pressure (A), and heart rate (B) from baseline in response to control (C), distension (D), acarbose (A) and combined (AD) treatments. Data are mean values ± SEM (n = 10). The $AUC_{0\text{-}120}$ for MAP was greater (A and AD compared to C; P = 0.005) with both acarbose treatments with no difference between C and D. The $AUC_{0\text{-}120}$ for HR was less (A and AD compared to C; P = 0.04) with both acarbose treatments with no difference between C and D.

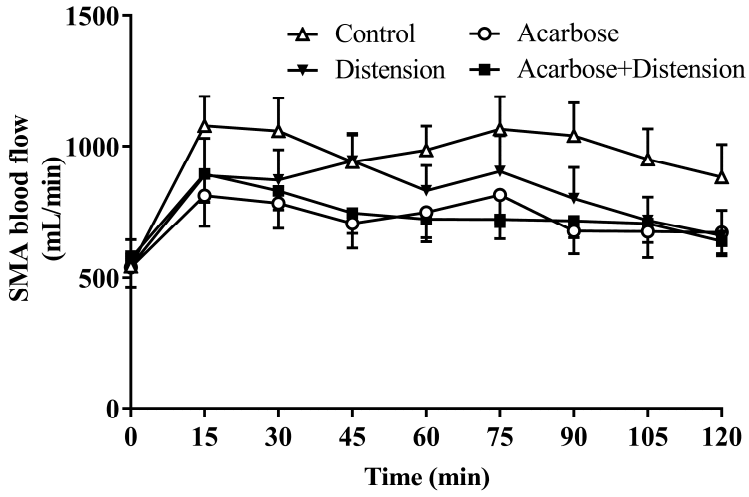

Figure 7.3. Superior mesenteric artery blood flow in response to control (C), distension (D), acarbose (A) and combined (AD) treatments. Data are mean values ± SEM (n = 9). The $AUC_{0\text{-}120}$ for SMA flow was less (A and AD compared to C; P = 0.003) with both acarbose treatments with no difference between C and D.

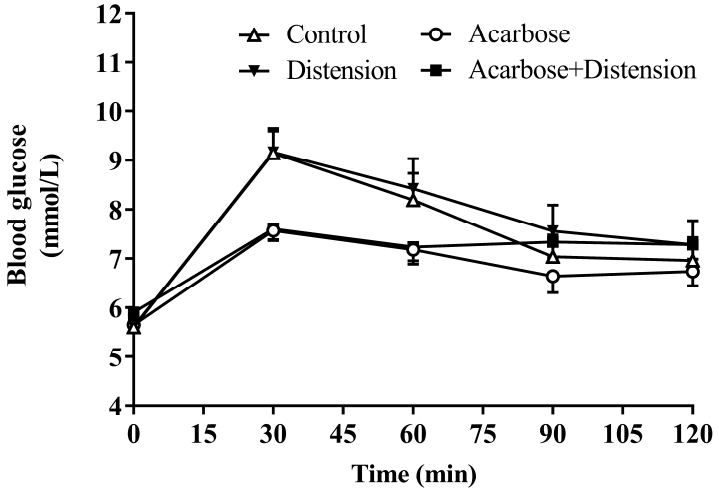

Figure 7.4. Blood glucose concentrations in response to control (C), distension (D), acarbose (A) and combined (AD) treatments. Data are mean values ± SEM (n = 9). The $AUC_{0\text{-}120}$ for blood glucose was reduced (A and AD compared to C; P = 0.03) by both acarbose treatments with no difference between C and D.

7.4 Discussion

This study evaluated the acute effects of acarbose and water drinking, alone and combined, on the BP and SMA blood flow responses to oral sucrose in healthy older adults. We demonstrated that in this group, ingestion of sucrose induced a substantial increase in SMA blood flow and fall in BP, as predicted [18, 107]. These changes were essentially abolished by acute administration of acarbose in a dose of 100mg; which was well tolerated. In contrast, water drinking alone (300 mL, 15 min before the sucrose load) to induce gastric distension had no significant effect on either BP or splanchnic blood flow and did not modify the response to acarbose.

This is the first study to evaluate the interaction between acarbose and gastric distension on 'postprandial' BP based on their potential for synergetic, or additive, effects. The observed responses to acarbose are consistent with previous reports, including our own [18, 107, 165, 166], and support the concept that acarbose will be useful in the management of PPH. We have further demonstrated that acarbose causes a profound attenuation of the rise in SMA blood flow induced by oral sucrose [107, 165] which is likely to be central to its anti-hypotensive effect. Such an effect was suggested in a previous study that was methodologically flawed [165]. The reduction in the splanchnic blood flow response to oral sucrose induced by acarbose is likely to reflect mechanisms unrelated to slowing of gastric emptying per se [18], given that the increase in SMA blood flow induced by ID administration of sucrose in healthy older adults is also markedly attenuated by acarbose [107]. The stimulation of GLP-1 as a result of the presence of nutrient in the more distal intestine may be relevant. In particular, we have demonstrated in healthy older adults that intravenous GLP-1 reduces the SMA blood flow response to ID glucose [106] and that the 'short-acting' GLP-1 agonist, lixisenatide, prevents the fall in systolic BP and reduces the rise in SMA flow following a 75g oral glucose load in

healthy older subjects and T2D patients [111]. However, in relation to a role for endogenous GLP-1, it would be expected that stimulation of GLP-1 would be associated with a concurrent equivalent increase in glucagon-like peptide-2 (GLP-2) [96, 448], which would be expected to stimulate SMA blood flow [114]. The reduction in plasma glucose induced by acarbose is predictably associated with a reduction in plasma insulin, which has vasodilatory properties [4].

We were surprised that water drinking had no significant effect on either BP or SMA blood flow. There are a number of potential explanations. We selected a relatively low volume (300 mL) drink to optimise tolerability, influenced by the outcome of our previous study demonstrating that this intragastric volume of water markedly attenuated the hypotensive response to intraduodenal glucose in healthy older adults [15]. With our study design, it would be anticipated that the majority of water would have been emptied from the stomach in the 15 minutes before the ingestion of the sucrose. Furthermore, the effects of gastric distension are known to be volume-dependent [90]. Hence, we cannot discount the possibility that a larger volume of water, given immediately before the sucrose may have been more effective. Moreover, the pressor effect of water drinking may also reflect changes in plasma osmolality [141, 142]. While there was a substantial fall in BP in response to sucrose, we studied healthy older adults, rather than patients with PPH, and none had evidence of autonomic neuropathy, although assessment of autonomic nerve function was indirect. The latter may also be of relevance to the negative outcome given that the pressor response to water appears to be exaggerated in patients with autonomic impairment [142]. It should also be appreciated that the number of subjects we studied was relatively small and there were non-significant trends for minor effects of water drinking soon after the sucrose drink. Accordingly, a type 2 error cannot be excluded. In addition, GLP-1 was not measured, which may have provided

mechanistic insights into the role of acarbose in modulating postprandial BP. A strength of our study was the inclusion of splanchnic blood flow measurement.

Previous studies have suggested that both acarbose and gastric distension may be potential approaches to reduce the postprandial fall in BP and our study aimed to evaluate the interaction between these interventions. The outcomes provide additional evidence to support the use of acarbose to attenuate the magnitude of the postprandial fall in BP, but not in combination with water drinking. The latter concept should, however, not be dismissed pending the outcome of further studies with greater subject numbers and particularly in patients with PPH and where water is consumed immediately prior to a meal.

Chapter 8

Effects of a guar and whey containing preload (Omniblend) on gastric emptying of, and the glycaemic, small intestinal absorption and blood pressure responses to, oral glucose in healthy older subjects

Statement of Authorship

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Name of Principal Author (Candidate)	Hung T Pham
Contribution to the Paper	Designed research and conducted research, analysed and interpreted data, wrote and revised paper.
Overall percentage	70%
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements

	with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.		
Signature		Date	Aug 2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Conceived and designed research, interpreted data and reviewed paper.		
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Contribution to the Paper	Conceived and designed research, interpreted data, reviewed paper and approved final version of paper.		
Signature		Date	Aug 2019

8.1 Introduction

The use of a macronutrient preload - a small amount of nutrient given 15-30 min before a main meal - to diminish postprandial glycaemic excursions, represents a novel approach to the management of type 2 diabetes (T2D) [149, 449-451]. The underlying rationale is that the presence of nutrients in the small intestine will stimulate the release of gut hormones, including glucagon-like peptide-1 (GLP-1), to augment insulin secretion and slow gastric emptying of the subsequent meal [452]. Gastric emptying, which exhibits a substantial inter-, but relatively low intra-individual, variation in health [326], is now recognised to be normal, or modestly accelerated, in the majority of people with uncomplicated T2D and relatively good glycaemic control [304]. In this group, slowing of gastric emptying is associated with a reduction in postprandial glucose [304], which is the major determinant of average glycaemic control, as assessed by HbA1c [304]. We have recently evaluated a preload containing whey (17g) and guar (5g), added to a 'shake and take' cup containing 150 mL water in participants with T2D [149]. Whey protein (a by-product of the cheese-making process) and guar gum (a viscous soluble fibre) have both been associated with slowing of gastric emptying [149, 451]. In these studies, the preload was well-tolerated when taken twice a day and substantially reduced the glycaemic response to a mashed potato meal, and modestly decreased glycated hemoglobin (0.1%) in T2D patients who were already well-controlled (mean HbA1c $6.6 \pm 0.1\%$), without inducing weight gain [149]. A subsequent study suggested that these effects were mediated predominantly by the whey protein; guar, in a dose of 5g, was less effective for lowering postprandial glucose compared with 17g whey [451]. In both studies, the reduction in postprandial glucose was associated with a tendency for a reduction in plasma insulin. Gastric emptying was assessed using an indirect stable isotope breath test technique in both studies, and reported to be slowed by the preload after both acute and 12 weeks' administration [149, 451]. However, a fundamental limitation of the breath test is that it cannot discriminate between

effects on gastric emptying from those on small intestinal glucose absorption, unlike the 'gold standard' technique of scintigraphy [453]; changes in small intestinal motility/absorption have been shown to have a major effect on postprandial glycaemia [454]. Accordingly, the effect of this preload on gastric emptying remains uncertain.

Preloads, including the guar/whey combination, also have potential to mitigate postprandial hypotension (PPH) – defined as a greater than 20 mmHg fall in systolic blood pressure (BP) after a meal, a condition which is associated with falls and syncope [1, 4], as well as increased mortality [68]. PPH currently lacks an effective treatment and occurs in up to 40% of patients with T2D [1, 5] and ~ 15% of the 'healthy' elderly [5, 74]. We have shown that the magnitude of the postprandial fall in BP is greater when gastric emptying is relatively more rapid in both healthy older subjects [70, 74] and patients with T2D [7], while slowing gastric emptying with guar gum attenuates the fall [22, 155]. Moreover, exogenous administration of GLP-1 [106] and the 'short-acting' GLP-1 receptor agonist, lixisenatide [111], both slow gastric emptying, and this is associated with marked attenuation in the magnitude of the fall in BP. Accordingly, a guar/whey preload has the potential to represent a simple, safe and inexpensive approach to the management of PPH.

The aims of this study were to determine the effects of the guar/whey preload on gastric emptying (measured with scintigraphy), intestinal glucose absorption and the glycaemic and BP responses to an oral glucose load in healthy older subjects.

8.2 Methods

8.2.1 Subjects

Eighteen healthy older subjects (7 male and 11 female, mean age 72.6 ± 1.1 years, BMI 26.3 ± 0.5 kg/m^2) participated in the study. All were non-smokers and none had a history of gastrointestinal disease or surgery, diabetes, significant respiratory, renal or cardiac disease, chronic alcohol abuse, epilepsy, or was taking medication known to influence BP or gastrointestinal function.

The study was registered at http://www.ANZCTR.org.au (ACTRN12619000438156), conducted in accordance with the Declaration of Helsinki, and approved by the Human Research Ethics Committee of the Royal Adelaide Hospital. Written informed consent was obtained from all participants.

8.2.2 Protocol

Each participant was studied on 2 occasions, separated by at least 7 days, in a randomised, crossover design. Randomisation was performed at the beginning of the first visit of each participant by an investigator tossing a coin. On each study day, participants attended the Clinical Research Facility, Adelaide Health and Medical Sciences building at The University of Adelaide at ~ 09.00 h after an overnight fast (14h for solids; 12h for liquids) [75]. They were seated with their back against a gamma camera (Siemens e.cam single-head gamma camera, Siemens Medical Solutions USA Inc, Knoxville, TN, USA), an intravenous cannula was inserted into an antecubital vein for blood sampling and an automated BP cuff placed around the opposite arm. After a 'rest period' of 15-30 minutes, to allow baseline BP to stabilise

[24], each participant was given either (i) a drink containing 50g glucose, 5g 3-O-methylglucose (3-OMG) and 20 MBq ^{99m}Tc-calcium phytate, made up to 300 mL with water [52] (the 'control' day) or (ii) a 'preload' containing 16.4g whey protein and 4.4g guar (90 kcal) (Vanilla flavour - GlucoControl^{TM}, Faulding; Omni Innovation, Campbellfield, VIC, Australia) made up to 150 mL with water 15 minutes before the drink (the 'preload' day). The preload and drink were both consumed within 2 minutes. Time zero (t = 0 min) was defined as the time of drink completion.

Measurements of gastric emptying, plasma glucose, plasma insulin, serum 3-OMG, superior mesenteric artery (SMA) blood flow, BP and heart rate (HR) and were obtained until t = 120 min. At the end of each study day, participants were offered a light lunch before they left the laboratory.

8.2.3 Measurements

8.2.3.1 Gastric emptying

Radioisotopic data were acquired in 1-min frames for the first 60 min and at 3-min frames thereafter up to 120 min. A region-of-interest was drawn around the total stomach and data were corrected for participant movement, radionuclide decay and γ-ray attenuation [7, 109]. Gastric emptying curves (expressed as % of the maximum content of the total stomach) were derived, and the intragastric content and at t = 0, 15, 30, 45, 60, 90 and 120 min and the 50% emptying time (T50) were calculated [298].

8.2.3.2 Plasma glucose and insulin

Venous blood samples (~10 mL) were obtained immediately prior to the ingestion of the preload (t = -18 min) and /or drink (t = -3 min) and then at t = 15, 30, 45, 60, 90 and 120 min. Samples were collected into ice-chilled EDTA-treated tubes and plasma was separated by centrifugation at 1996 ×g for 15 minutes at 4°C within 15 minutes of collection and stored at −80°C until assayed [455].

Plasma glucose was measured using the hexokinase technique (2900D Biochemistry Analyzer, YSI Incorporated, Yellow Springs, Ohio, USA) [455]. Plasma insulin was measured by ELISA (Diagnostics 10-1113, Mercodia, Uppsala, Sweden). The sensitivity of the assay is 1.0 mU/L and intra- and inter-assay coefficients of variation are 2.9% and 6.7%, respectively [455].

The insulinogenic index (the ratio of change in insulin over change in glucose over the first 30 min after the glucose drink) was calculated to estimate early-phase insulin secretion [456].

8.2.3.3 Oral glucose absorption (serum 3-OMG)

Venous blood samples (~5 mL) were collected into untreated tubes and allowed to clot. Serum was separated by centrifugation at 1996 ×g for 15 minutes at 4°C and stored at −80°C until assayed [455]. Serum 3-OMG concentrations were measured by liquid chromatography and mass spectrometry, with a sensitivity of 0.0103 mmol/L [457].

8.2.3.4 Superior mesenteric artery blood flow

Superior mesenteric artery (SMA) blood flow (mL/min) was measured immediately prior to the ingestion of the preload (t = -18 min) and/or drink (t = -3 min), and then at t = 15, 30, 45,

60, 90 and 120 min using a Logiq e ultrasound system with a 3.5C broad-spectrum 2.5–4 MHz convex linear array transducer (GE Healthcare Technologies, Sydney, NSW, Australia) [366].

8.2.3.5 Blood pressure and heart rate

Systolic and diastolic BP (SBP and DBP) and HR were measured with an automated oscillometric BP monitor (DINAMAP ProCare 100, GE Medical Systems, Milwaukee, WI, USA) at 3-min intervals prior to administration of the drink, and then at 5-min intervals from t = 0 - 120 min. Fasting BP and HR were calculated as an average of measurements obtained at t = -24, -21, -18 min prior to the ingestion of the preload (t = -17 min), or at t = -9, -6, -3 min prior to the ingestion of the drink (t = -2 min) on the study day without a preload. PPH was defined as a fall in SBP ≥ 20 mmHg that was sustained for ≥ 30 min [7].

8.2.3.6 Cardiovascular autonomic nerve dysfunction

Standardised cardiovascular reflex tests were used to assess autonomic nerve function [445-447]. Parasympathetic function was evaluated by the variation (R-R interval) of HR during deep breathing and the immediate response to standing ("30:15" ratio). Sympathetic function was assessed by the decrease in SBP in response to standing. Each result was scored according to age-adjusted predefined criteria: 0 = normal, 1 = borderline and 2 = abnormal, for a maximum total score of 6. A score ≥ 3 was considered to indicate autonomic dysfunction [447].

8.2.4 Statistical analysis

Gastric emptying, plasma glucose and insulin, serum 3-OMG, SMA blood flow, SBP, DBP and HR were analysed and presented as absolute values. Two-way repeated-measures analysis of variance (ANOVA) with treatment and time as factors, with Bonferroni's correction for post

hoc comparisons, was used to analyse gastric emptying, plasma glucose, plasma insulin, serum 3-OMG and SMA blood flow. One-way repeated-measures ANOVA was used to evaluate the effect of time on SBP, DBP and HR. The maximum falls in SBP and DBP and increases in HR, plasma glucose and insulin, serum 3-OMG and SMA blood flow were defined as the greatest change from t = -3 min in each subject at any given time point on each study day. Areas under the curves (AUCs) for SBP, DBP, HR, and serum 3-OMG were calculated. Incremental areas under the curves (iAUCs) were calculated for plasma glucose and insulin. AUCs for SBP, DBP and HR, the insulinogenic index and maximum increases/falls in the two study days were compared using Student's paired t-test. Pearson's correlations were used to evaluate relationships between plasma glucose, plasma insulin and serum 3-OMG between the two study days. All analyses were performed using SPSS version 24 (SPSS, Chicago, IL, USA); a P-value < 0.05 was considered significant. Data are shown as mean values ± SEM.

8.3 Results

The studies were generally well tolerated. No participant had autonomic nerve dysfunction. In one participant, extensive small intestinal overlap precluded acceptable gastric emptying analysis and the scintigraphic data were not included. In another subject, 3-OMG was inadvertently not included in the drink on one of the two days. Accordingly, paired BP, HR, SMA blood flow, plasma glucose and insulin data were available in 18 subjects, while gastric emptying and serum 3-OMG data were available in 17 subjects.

8.3.1 Gastric emptying

Gastric emptying of the glucose drink approximated an overall linear pattern (time effect: P < 0.001, Figure 8.1). There was no difference in gastric retention evaluated by ANOVA

(treatment effect: P = 0.96, treatment × time interaction: P = 0.24, Figure 8.1), nor T50 (preload: 76.5 ± 4.3 vs control: 76.5 ± 4.8 min, P = 0.99). At t = 120min, gastric emptying was incomplete in all subjects on both days.

8.3.2 Plasma glucose and insulin

There were no differences in baseline (fasting) plasma glucose or insulin between the two days (Table 8.1). After ingestion of the preload, there was no change in plasma glucose between t = -18 min and t = -3 min (5.2 ± 0.1 vs 5.0 ± 0.2 mmol/L, P = 0.26), while plasma insulin increased (5.3 ± 0.8 vs 7.0 ± 1.1 mU/L, P = 0.02).

There was a rise in plasma glucose on both days following the glucose drink (time effect: P < 0.001, Figure 8.2A). Plasma glucose concentrations were lower after the preload compared to control (treatment effect: P = 0.02), with significant differences at t = 30, 45 and 60 min (P < 0.05 for each, Figure 8.2A). The maximum rise in plasma glucose after the glucose drink was also less after the preload (preload: 3.7 ± 0.4 vs control: 4.3 ± 0.3 mmol/L, P = 0.007).

There was a rise in plasma insulin on both days following the glucose drink (time effect: P < 0.001, Figure 8.2B). Plasma insulin concentrations were greater after the preload (treatment x time interaction: P = 0.03) at t = 15 and 30 min (P < 0.001 for each, Figure 8.2B). There was no difference in the maximum rise in plasma insulin after the glucose drink between the two days (preload: 63.1 ± 8.5 vs control: 62.8 ± 11.8 mU/L, P = 0.97).

The insulinogenic index at 30 min was higher after the preload, compared with control (preload: 18.1 ± 2.4 vs control: 10.9 ± 1.5, P = 0.002).

8.3.3 Glucose absorption

There was an increase in serum 3-OMG on both the preload and control study days following the glucose drink (time effect: $P < 0.001$, Figure 8.2C). Serum 3-OMG concentrations were lower after the preload (treatment effect: $P = 0.003$ and treatment x time interaction: $P = 0.002$) with significant differences at t = 45, 60, 90 and 120 min ($P < 0.05$ for each, Figure 8.2C). The peak serum 3-OMG concentrations were also less after the preload (preload: 0.84 ± 0.04 vs control: 0.92 ± 0.03 mmol/L, $P = 0.01$).

8.3.4 Superior mesenteric artery blood flow

There was no difference in baseline (fasting) SMA blood flow between the two study days (Table 8.1). Following the preload, there was no change in SMA blood flow between t = -17 min to t = -2 min (329 ± 24.8 vs 371 ± 27.5 mL/min, $P = 0.10$).

There was an increase in SMA blood flow on both days following the glucose test drink (time effect: $P < 0.001$ for all, Figure 8.3). This rise was attenuated after the preload (treatment effect: $P < 0.001$ and treatment x time interaction: $P = 0.003$) with significant differences at t = 15, 60, 90 min ($P < 0.05$ for each, Figure 8.3). The maximum increase in SMA flow was also less following the preload (preload: 365.5 ± 29.4 vs control: 488.2 ± 42.5 mL/min, $P = 0.002$).

8.3.5 Blood pressure and heart rate

There were no differences in baseline (fasting) SBP, DBP and HR between the two days (Table 8.1). Following ingestion of the preload, there was a trend for an increase in SBP (120.0 ± 3.2 vs 124.3 ± 3.9 mmHg, $P = 0.06$), with no change in DBP (67.3 ± 1.9 vs 69.2± 2.1 mmHg, $P = 0.22$) or HR (63.1 ± 2.3 vs 63.1 ± 2.4 bpm, $P = 0.17$) from t = -18 min to t = -3 min. No

participant had PPH, i.e. a sustained fall in SBP of more than 20mmHg for 30 min, compared to their fasting measurement on either day.

8.3.5.1 Systolic blood pressure

There was a modest fall in SBP on both days (P = 0.001 for both; Figure 8.4A), without any difference in the $AUC_{0\text{-}120min}$ for SBP (P = 0.98), or the maximum fall in SBP (preload: -13.2 ± 2.5 vs control: -12.4 ± 1.7 mmHg; P = 0.77) between the two days.

8.3.5.2 Diastolic blood pressure

There was a modest fall in DBP on both days (P < 0.01 for both; Figure 8.4B), without any difference in the $AUC_{0\text{-}120min}$ for DBP (P = 0.55) or the maximum fall in DBP (preload: -11.4 ± 1.4 vs control: -11.1 ± 1.1 mmHg; P = 0.82) between the two days.

8.3.5.3 Heart rate

There was a modest increase in HR on both the preload (P = 0.001) and control (P = 0.009) days (Figure 8.4C). The $AUC_{0\text{-}120min}$ for HR was greater (P = 0.007) on the preload day. The maximum rise in HR after the glucose drink also tended to be greater after the preload (preload: 11.3 ± 2.0 vs control: 7.9 ± 1.0 bpm, P = 0.08).

8.3.6 Relationships between plasma glucose and insulin or serum 3-OMG between the two study days

Between t = 0 - 120 min, there was a correlation between the difference in iAUCs for plasma glucose and the difference in AUCs for serum 3-OMG between the preload and control days

(R = 0.71, P = 0.002, Figure 8.5). However, there was no correlation between the difference in iAUCs for plasma glucose and the difference in iAUCs for plasma insulin between the preload and control days (R = 0.31, P = 0.21).

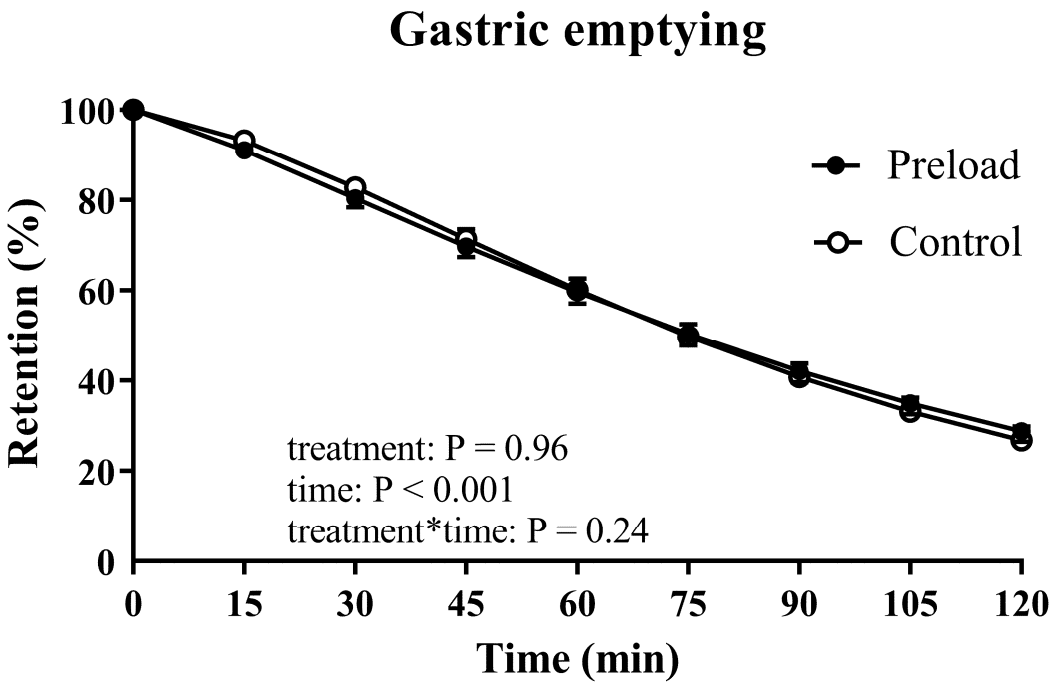

Figure 8.1. Gastric retention following ingestion of 50 g glucose in 300 mL water on the control and preload days. Results of repeated measures ANOVA are reported as P values for differences by treatment (treatment), differences over time (time) and differences due to the interaction of treatment and time (treatment*time). Data are mean ± SEM (n = 17).

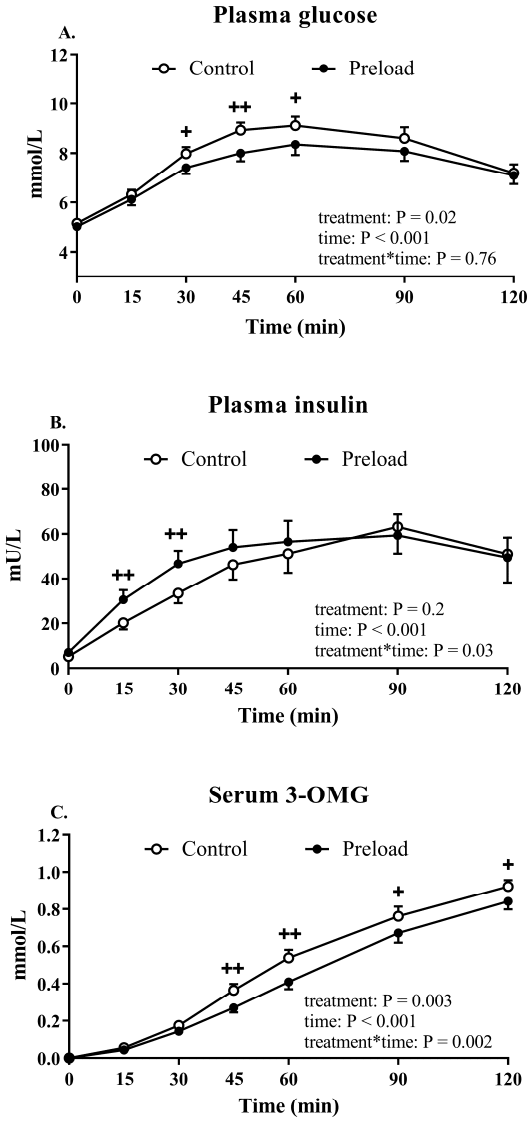

Figure 8.2. Plasma glucose (A), plasma insulin (B) and serum 3-OMG (C) on the control and preload days. Results of ANOVA are reported as P values for differences by treatment (treatment), differences over time (time) and differences due to the interaction of treatment and time (treatment*time). Post hoc comparisons, adjusted by Bonferroni's correction, were performed, if ANOVA values (treatment*time) were significant. + $P < 0.05$ and ++ $P < 0.01$. Data are mean values ± SEM (n = 18 for plasma glucose and insulin and n = 17 for 3-OMG).

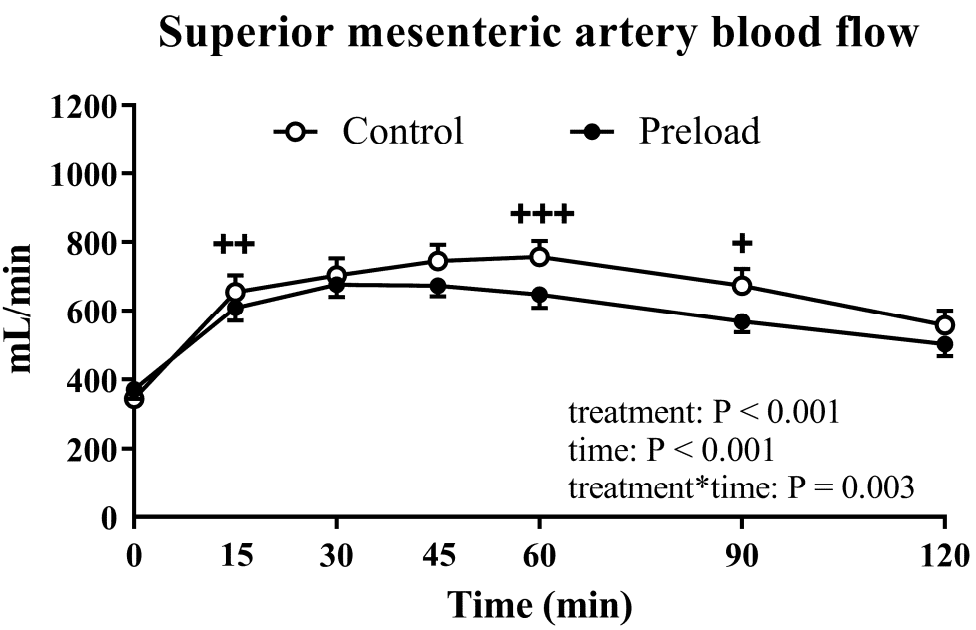

Figure 8.3. Superior mesenteric artery blood flow on the control and preload days. Results of ANOVA are reported as P values for differences by treatment (treatment), differences over time (time) and differences due to the interaction of treatment and time (treatment*time). Post hoc comparisons, adjusted by Bonferroni's correction, were performed, if ANOVA values (treatment*time) were significant. + $P < 0.05$, ++ $P < 0.01$, +++ $P < 0.001$. Data are mean values ± SEM (n = 18).

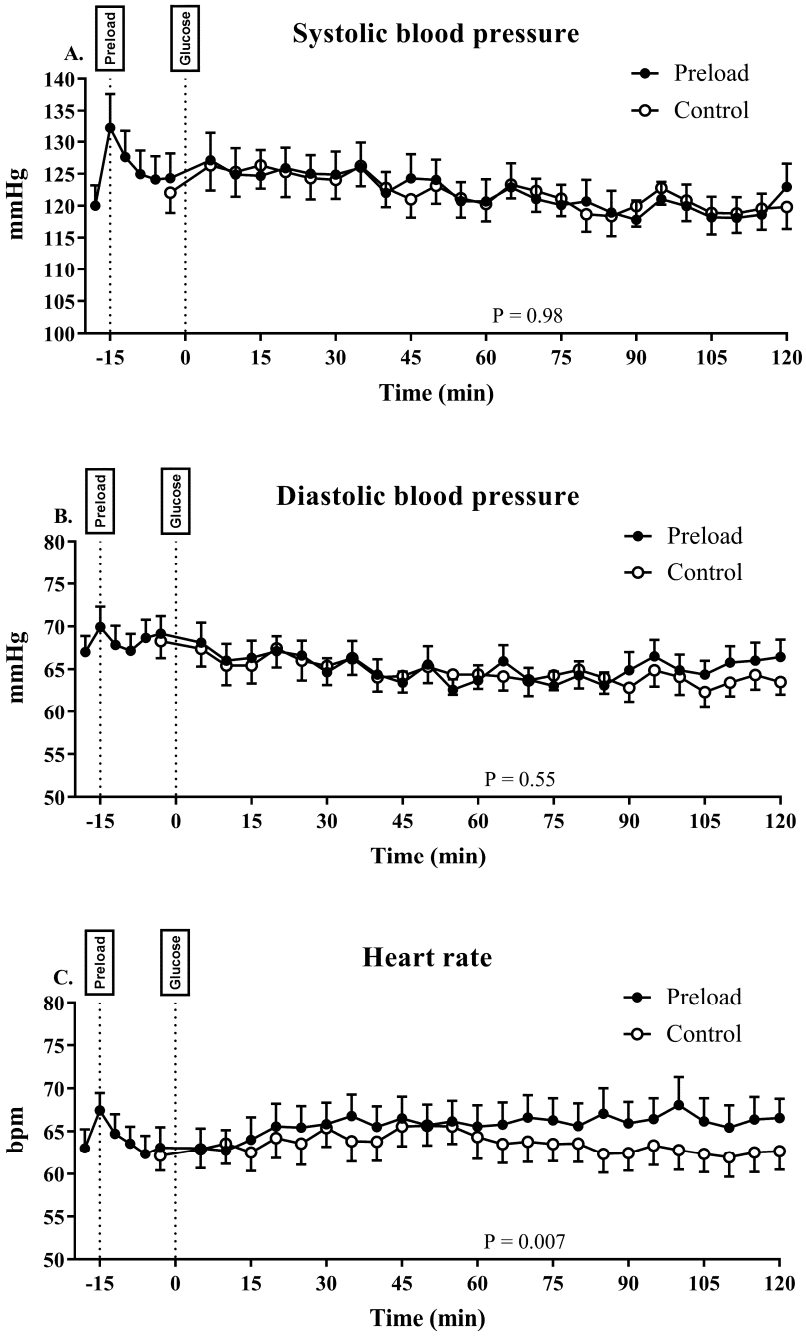

Figure 8.4. Systolic (A) and diastolic BP (B) and heart rate (C) on the control and preload days. Data are mean values ± SEM (n = 18).

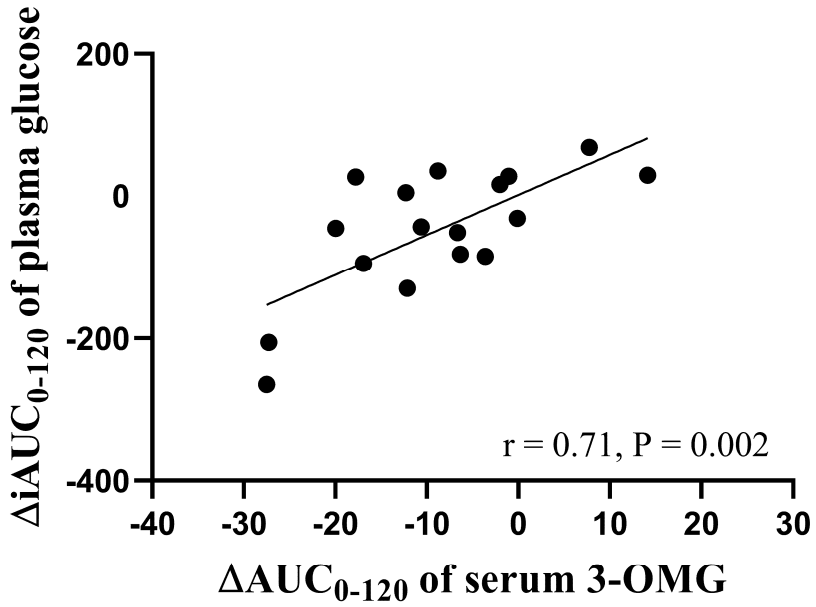

Figure 8.5. Relationship between the difference in iAUCs for plasma glucose between the preload and control days with the difference in AUCs for serum 3-OMG between the preload and control days (n = 17).

8.4 Discussion

Our study has demonstrated that a guar/whey preload diminishes the glycaemic response to a glucose drink in healthy older subjects, as previously demonstrated in participants with T2D [149, 449]. However, this effect was not associated with slowing of gastric emptying. There was a substantial delay in small intestinal glucose absorption, and stimulation, rather than a reduction, in insulin secretion. Moreover, changes in glycaemia and glucose absorption were related. The preload had no effect on the reduction in BP following the glucose load, but the latter was modest, and no subject had PPH. In contrast, the rise in SMA blood flow was attenuated by the preload.

The pivotal importance of upper gastrointestinal function to postprandial glycaemia in health and diabetes is now widely appreciated [458, 459]. The focus has hitherto been on gastric emptying, which is frequently disordered in diabetes [71], albeit usually normal, or modestly accelerated, in uncomplicated T2D patients [304]. Postprandial glycaemic excursions are a major determinant of glycated haemoglobin, particularly when the latter is ≤ 8.0% [149], and can be diminished by slowing gastric emptying, which has stimulated the development of dietary and pharmacological strategies to target this mechanism. Examples of the latter are the amylin agonist, pramlintide [312] and 'short-acting' GLP-1 receptor agonists [460]. Our previous studies suggested that a guar/whey 'preload' reduced postprandial glycaemia in T2D predominantly by slowing gastric emptying [149, 451]. However, the latter was assessed using a stable isotope breath test technique, which cannot discriminate effects on gastric emptying from those on small intestinal carbohydrate absorption. In the current study, the preload reduced glycaemia substantially, particularly given that healthy subjects were studied, rather than those with T2D, but had no effect on gastric emptying; indeed, the gastric emptying curves were essentially superimposed.

It should be appreciated that the preload (90 kcal) was taken 15 min before the drink and, because gastric emptying is usually in the range of 1-4 kcal/min [220, 221], would not be expected to have emptied completely from the stomach at the time of ingestion of the drink. Furthermore, at 120 min, the drink has not emptied from the stomach completely. Accordingly, we cannot exclude the possibility that, because of its higher intragastric caloric content, after the preload, while the rate of gastric emptying of the drink was unaffected between 0-120min, the drink may have taken longer to empty completely. A reduction in small intestinal glucose absorption is shown unequivocally by the observed marked reduction in the rate of 3-OMG absorption in the absence of any difference in gastric emptying and is, accordingly, likely to contribute to glucose-lowering. This concept is also supported by the observed relationship between the changes in plasma glucose and 3-OMG. In contrast to previous studies [149, 461], the preload was associated with an increase in plasma insulin, which is likely to reflect stimulation of insulin secretion by amino acids in the whey protein [451, 452]. Slowing of gastric emptying has the capacity to override insulinotropic responses, as shown by the reduction in postprandial insulin induced by exogenous administration of GLP-1 [285, 462].

Our study also evaluated the potential for the guar/whey preload in the management of PPH. Both oral (9g) [155] and intraduodenal (4g) [23] administration of guar diminish the hypotensive response to carbohydrates. There was, however, only a modest fall in BP in response to oral glucose, presumably because participants were all healthy, and no effect of the preload on BP. In contrast, the preload attenuated the rise in SMA blood flow substantially, presumably secondary to the reduction in glucose absorption. The small rise in HR after the preload might be attributable to the sympathetic effect of insulin [463, 464]. Altogether, further studies in patients with PPH are indicated before the approach is dismissed.

In interpreting our observations, specific limitations should be appreciated: the study was not placebo-controlled, the size of the cohort was relatively small, healthy older subjects rather than T2D were studied, and responses to oral glucose, rather than a meal, were evaluated. Moreover, we did not measure GLP-1 or glucose dependent insulinotropic polypeptide (GIP) levels.

In conclusion, the reduction in the glycaemic response to oral glucose by a guar/whey preload in healthy older subjects reflects a slowing of small intestinal glucose absorption and stimulation of insulin secretion, rather than a delay in gastric emptying.

Table 8.1. Baseline (fasting) measurements in the subjects (n = 18)[1]

	Control (t = -3)	Preload (t = -18)	P value
SBP (mmHg)	122.1 ± 3.2	120.0 ± 3.1	0.10
DBP (mmHg)	68.2 ± 1.9	67.3 ± 1.9	0.38
HR (beats/min)	62.1 ± 1.7	63.9 ± 2.3	0.09
Plasma glucose (mmol/L)	5.2 ± 0.1	5.2 ± 0.1	0.68
Plasma insulin (mU/L)	5.2 ± 0.9	5.3 ± 0.8	0.68
SMA blood flow (mL/min)	344 ± 23.6	329 ± 24.8	0.34

[1] All values are absolute values. Differences between study days were tested via paired t-tests. Values are means ± SEMs. SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; SMA, superior mesenteric artery.

Chapter 9

Longitudinal changes in fasting and glucose-stimulated GLP-1 and GIP in healthy older subjects

Statement of Authorship

Title of paper	Longitudinal changes in fasting and glucose-stimulated GLP-1 and GIP in healthy older subjects.
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Principle Author

Name of Principal Author (Candidate)	Hung T Pham
Contribution to the Paper	Conducted research, analysed and interpreted data, wrote and revised paper.
Overall percentage	70%
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the primary author of this paper.

Signature		Date	Aug 2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Signature		Date	Aug 2019

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Contribution to the Paper	Interpreted data and reviewed paper.		
Signature		Date	Aug 2019

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Contribution to the Paper	Interpreted data and reviewed paper.		
Signature		Date	Aug 2019

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Contribution to the Paper	Interpreted data and reviewed paper.		
Signature		Date	Aug 2019

Name of Co-Author	Michael Horowitz		
Contribution to the Paper	Conceived and designed research, interpreted data and reviewed paper.		
Signature		Date	Aug 2019

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Contribution to the Paper	Conceived and designed research, interpreted data, reviewed paper and approved the final version of paper.		
Signature		Date	Aug 2019

9.1 Introduction

The incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), released from the small intestine in response to nutrient exposure (GLP-1 from 'L' cells located predominantly in the distal small intestine and GIP from 'K' cells located more proximally) are important regulators of postprandial glycaemia through glucose-dependent insulinotropic effects [297]. They account for the so-called 'incretin effect' - the markedly increased insulin response when glucose is delivered enterally when compared with an isoglycaemic intravenous infusion [279, 465]. There is a substantial inter-individual variation in GLP-1 and GIP responses to nutrients [466], which is probably not systematically affected by the presence of type 2 diabetes (T2D) [466]. GLP-1 impacts on glucose homeostasis through a number of actions, in addition to stimulation of insulin secretion, including suppression of glucagon, slowing of gastric emptying and enhanced satiety to reduce food intake [119, 467]. In contrast, GIP, which may stimulate glucagon, particularly in response to hypoglycaemia [468], has little, or no, effect on gastric emptying or appetite [282, 468], but may affect fat metabolism [119, 469]. In T2D the incretin effect is diminished, at least in part, as a result of a marked attenuation of the insulinotropic effect of GIP [289]. This observation has stimulated the development of GLP-1-based therapy – ie. GLP-1 receptor agonists and dipeptidyl peptidase 4 (DPP-4) inhibitors – for the management of T2D [311, 470].

It is now appreciated that the rate of gastric emptying, which varies substantially between individuals (usually in the range of 1-4 kcal/min), is a major determinant of the glycaemic response to carbohydrate-containing meals in healthy subjects [251, 298, 299, 301, 471] as well as individuals with T2D [7, 472]. Gastric emptying may also impact on postprandial GLP-1 and GIP secretion [326]. We have demonstrated that the rate of intraduodenal (ID) glucose delivery has differential effects on GLP-1 and GIP in health [305, 306, 308] and type 2 diabetes

[308, 455]. Specifically, a rate of > 2kcal/min is required to stimulate GLP-1, whereas the stimulation of GIP is approximately linear in the rage of 1-4 kcal/min [305, 306, 326]. Using the specific GLP-1 antagonist, exendin 9-39, we have also shown that endogenous GLP-1 slows gastric emptying [473].

While gastric emptying is known to slow modestly with healthy ageing [70, 249], it is not known whether baseline and/or nutrient-stimulated GLP-1 or GIP levels are subject to intra-individual variation or whether they are affected by ageing. Specifically, previous studies have all been cross-sectional in design, with the inherent limitations in this approach. We have now re-evaluated a cohort of healthy older subjects after an interval of ~ 5.9 years to evaluate changes in fasting and glucose-stimulated plasma GLP-1 and GIP concentrations, and their relationships with gastric emptying.

9.2 Materials and methods

9.2.1 Subjects

87 healthy older individuals who took part in a study, performed between July 2010 and July 2012, evaluating the effect of gastric emptying on the glycaemic and incretin hormone responses to a 75g oral glucose load [299] were invited by mail to participate in the present follow-up study. Of the original cohort, none had died, 41 agreed to participate, 11 had medical conditions that precluded their involvement, 13 refused to participate, 21 did not respond to the letter and, in 1 case, the invitation letter was returned and the individual considered to be lost to follow-up. Demographic information and medical history were updated. Individuals were excluded if they had a history of gastrointestinal disease or surgery, significant respiratory or

cardiac disease, alcohol intake > 20g/day or were taking medication known to affect gastric emptying.

The protocol was approved by the Human Research Ethics Committee of the Royal Adelaide Hospital, and each subject provided written informed consent. All experiments were carried out in accordance with the Declaration of Helsinki.

9.2.2 Protocol

The protocol was identical to that used in the initial study [299]. Individuals attended the Royal Adelaide Hospital, at ~08.30 h after an overnight fast (14 h for solids; 12 h for liquids) [75]. They were seated in an arm-chair, and a cannula was inserted into an antecubital vein for blood sampling. After a 'rest period' of 15-30 min, each participant consumed a drink containing 75g glucose and 150mg ^{13}C-acetate (Cambridge Isotope Laboratories, Tewksbury, MA, USA), made up to 300mL with water, within 2 minutes. Time zero (t = 0) was defined as the time of completion of the drink.

Venous blood samples (~ 15 mL) were obtained immediately prior to the commencement of the drink (t = −3 minutes) and at t = 30, 60, 90 and 120 min. The intravenous cannula was then removed and the subject offered a light lunch before leaving the laboratory.

9.2.3 Measurements

9.2.3.1 Blood glucose concentrations

Fasting and two-hour blood glucose concentrations were determined using a portable glucometer (Medisense Companion 2 Meter, Medisense Inc., Waltham, MA, USA) [434, 435].

Subjects with fasting blood glucose ≥7.0 mmol/L and/or 2h blood glucose ≥11.1 mmol/L were classified, according to WHO criteria, as having diabetes [474].

9.2.3.2 Plasma GLP-1 and GIP

Total GLP-1 was measured by radioimmunoassay (GLPIT-36HK, Millipore, Billerica, MA, USA). The minimum detectable limit was 3 pmol/L, intra- and inter-assay CVs were 8.0% and 10.0% respectively [306]. Plasma GIP was measured by radioimmunoassay. The minimum detectable limit was 2 pmol/L, interassay CV was 9.4% and intra-assay CV was 4.4% [475]. The assays used for the initial and follow-up measurements were identical, although they were not performed at the same time.

9.2.3.3 Gastric emptying

Exhaled breath samples were collected before ingestion of the drink (t = -3 min), every 5 minutes for the first hour (commencing at t = 5 min) and then every 15 minutes for the second hour, for assessment of gastric emptying. The $^{13}CO2$ concentration in the breath samples was measured by an isotope ratio mass spectrometer (ABCA 20/20; Europa Scientific, Crewe, UK), and the gastric 50% emptying time (T50) was calculated, using the formula described by Ghoos et al. [264].

9.2.4 Statistical analysis

Plasma GLP-1 and GIP were analysed and presented as absolute values and changes from baseline. One-way repeated-measures ANOVA was used to evaluate the effects of time (t = 0-120 mins) on the change from baseline values for plasma GLP-1 and GIP, at each visit.

The peak and time to peak for GLP-1 and GIP were calculated, as well as areas under the curve (AUCs) from 0 – 120 min, the latter using the trapezoidal rule. Differences between the initial study and follow-up were assessed using Student's paired t-tests.

The changes in AUCs for plasma GLP-1 (Δ.GLP-1.AUC), plasma GIP (Δ.GIP.AUC) and gastric emptying (ΔGE) between the initial and follow-up studies were calculated. Linear regression analysis was performed to evaluate correlations for GLP-1 and GIP concentrations between the two study days. Multiple linear regression was performed to gain insights into the potential effects of ΔGE, GLP-1 and GIP responses at the initial study, and BMI at the initial study on the changes in GLP-1 or GIP. All analyses were performed using SPSS version 24 (SPSS, Chicago, IL, USA). A P value < 0.05 was considered significant in all analyses. Data are presented as mean values ± SEM, unless stated otherwise

9.3 Results

Of the 41 older individuals (20 female and 21 male) who agreed to return, the mean age at the initial study was 71.2 ± 3.8 (SD) years and body mass index [BMI] 25.8 ± 2.7 (SD) kg/m^2. At 'follow-up' (mean interval of 5.9 ± 0.1 years), age was 77.1 ± 3.8 (SD) years and body mass index [BMI] 26.5 ± 3.1 (SD) kg/m^2 (P = 0.3). There were no demographic differences at baseline between those who participated in the study and those who did not. The studies were generally well tolerated and there were no adverse events. Five subjects were shown to have diabetes and were excluded from analyses. In another 6 subjects, a nonlinear regression model could not be fitted to the measured ^{13}CO2 concentrations at the initial and/or the follow-up study. Paired plasma GLP-1 and GIP data were available in 36 subjects, while gastric emptying data were available in 30 subjects.

9.3.1 Plasma GLP-1

There was a reduction in fasting plasma GLP-1 (21.0 ± 1.0 vs 15.3 ± 0.7 pmol/L, P < 0.001) at follow-up compared to the initial study. Between t = 0 and 120 min, there was a rise (P < 0.001 for both) in plasma GLP-1 on both study days. The AUC for plasma GLP-1 (P = 0.001) (Figure 9.1A) and the maximum rise in plasma GLP-1 (41.3 ± 2.6 vs 35.4 ± 2.2 pmol/L, P = 0.03) were less, and the time to peak was longer (33.4 ± 2.0 vs 47.5 ± 3.6 min, P = 0.001), at follow-up. Changes in GLP-1 were not influenced by gender (data not shown).

9.3.2 Plasma GIP

There was a reduction in fasting plasma GIP (19.8 ± 1.2 vs 17.1 ± 0.9 pmol/L, P = 0.03) at follow-up compared to the initial study. Between t = 0 and 120 min, there was a rise (P < 0.001 for both) in plasma GIP on both study days. There was no difference (P = 0.26) in the AUC for plasma GIP between the initial and follow-up studies (Figure 9.1B), or in the maximum rise in plasma GIP (56.1 ± 2.8 vs 54.2 ± 2.4 pmol/L, P = 0.43). However, the time to peak was shorter (98.3 ± 3.7 vs 76.7 ± 5.9 min, P = 0.001) at follow-up. Changes in GIP were not influenced by gender (data not shown).

9.3.3 Gastric emptying

Gastric emptying (T50) was slower at follow-up than at the initial study (136.5 ± 4.9 vs 164.7 ± 10.6 min, P = 0.008).

9.3.4 Relationships for GLP-1, GIP and gastric emptying between the two study days

Fasting GLP-1 concentrations at the initial and follow-up studies were not related (R = 0.23, P = 0.18; n = 36). In contrast, there was a correlation between but fasting GIP concentrations

between the initial and follow-up days (R = 0.72, P < 0.001; n = 36). There were significant relationships between $AUC_{0\text{-}120min}$ for plasma GLP-1 (R = 0.50, P = 0.002; n = 36) (Figure 9.2A), plasma GIP (R = 0.60, P < 0.001; n = 36) (Figure 9.2B) and gastric emptying (R = 0.38, P = 0.04; n = 30) at the initial and follow-up studies.

9.3.5 Predictors of changes in glucose-stimulated plasma GLP-1 and GIP

Multiple linear regression analysis was performed to investigate potential predictors of the Δ.GLP-1.AUC in the subjects with complete data (n = 30). Variables in the model included the ΔGE between the two study days, GLP-1.AUC at the initial study and BMI at the initial study. GLP-1.AUC at the initial study (β = -0.47 ± 0.17 (SE), P = 0 .01), but not ΔGE (β = 1.5 ± 3.4 (SE), P = 0.96) or BMI at the initial study (β = 35.5 ± 62.0 (SE), P = 0.57), was a significant predictor. The overall model fit was $r^2 = 0.26$, P = 0.046.

Similarly, multiple linear regression analysis was also performed to investigate potential predictors of the Δ.GIP-1.AUC in the subjects with complete data (n = 30). Variables in the model included the ΔGE between the two study days, GIP.AUC at the initial study and BMI at the initial study. GIP.AUC at the initial study (β = -0.48 ± 1.4 (SE), P = 0.002), but not ΔGE (β = 0.38 ± 4.7 (SE), P = 0.94) or BMI at the initial study (β = 37.8 ± 93.2 (SE), P = 0.69), was a significant predictor. The overall model fit was $r^2 = 0.33$, P = 0.02.

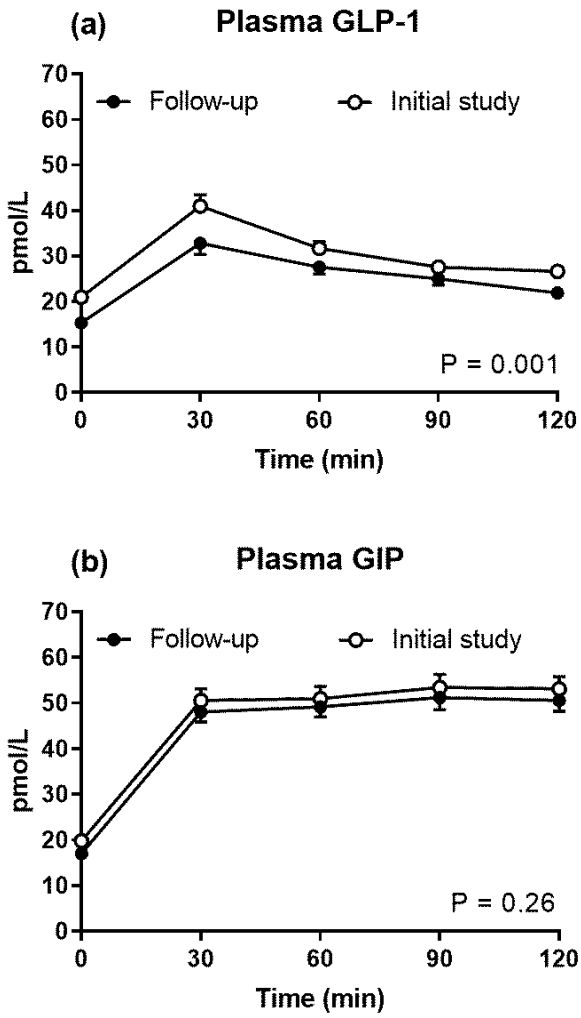

Figure 9.1. Plasma (A) GLP-1 and (B) GIP concentrations before and after 75g glucose at the initial and follow-up studies in healthy older subjects (n = 36). Data are mean values ± SEM.

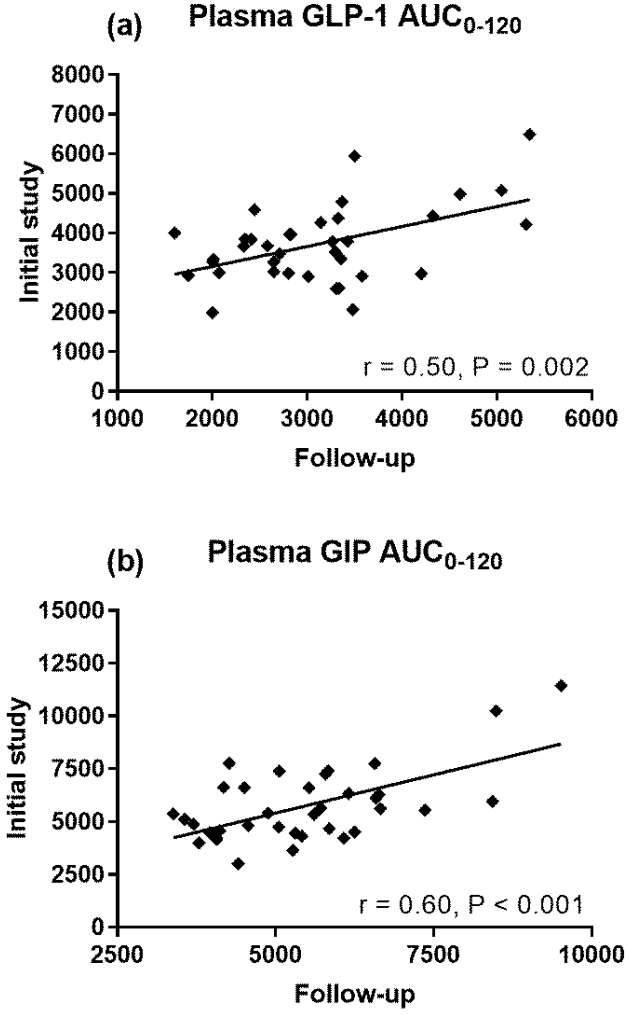

Figure 9.2. Relationship between $AUC_{0\text{-}120min}$ for plasma (A) GLP-1 and (B) GIP at the initial study and follow-up (n = 36).

9.4 Discussion

To our knowledge, this study represents the first longitudinal evaluation of fasting and oral glucose-stimulated incretin hormone responses in healthy older people. Previous studies have all been cross-sectional. Novel observations are that fasting GIP and GLP-1, and glucose-stimulated GLP-1 decreased significantly over a mean period of ~5.9 years and there was a reasonable correlation between GLP-1 and GIP responses at baseline and at follow-up. Neither the slight slowing of gastric emptying nor BMI at the initial study predicted the changes in GLP-1 or GIP.

Existing information relating to the effects of ageing on incretin hormones in healthy people and patients with T2D is inconsistent. Some cross-sectional studies have reported that fasting and/or 'postprandial' plasma GIP [476-478] and GLP-1 [256, 478, 479] concentrations do not differ between healthy young and older subjects. However, in other studies, postprandial GIP [480, 481] and GLP-1 [478, 480, 482] were reported to be slightly greater in healthy older subjects. In T2D, postprandial GIP has been reported to be greater [476, 483] or comparable [477], while GLP-1 has been reported to be less in older subjects with T2D [477]. The fundamental limitation of these cross-sectional studies is the inability to account for intra-individual changes over time.

Our study indicates that ageing is associated with modest reductions in fasting GLP-1 and GIP, and oral glucose-stimulated GLP-1, but not GIP. The pathophysiology underlying the changes in fasting and postprandial incretin hormones is uncertain. Our focus was the incretin secretory responses and we, accordingly, measured plasma concentrations of total, rather than intact, GLP-1 and GIP which include both intact hormones and inactive metabolites [465].

The relevance of the observed changes is also uncertain. However, Færch et al. have suggested that a reduction in the GLP-1 response to oral glucose could predispose to the development of T2D [484]. This is supported by recent longitudinal observations in 121 subjects (non-diabetic lean and obese adult men and women) in the Hoorn Meal Study in which a reduced GLP-1 response in an oral glucose tolerance test was associated with a greater increase in fasting glucose 7 years later [485], although an oral glucose tolerance test was not performed at follow-up, and gastric emptying was not studied. Nevertheless, our data add to the concern that a reduction in fasting and glucose-stimulated GLP-1 may predispose to impairment in glucose tolerance and T2D.

We observed strong correlations in fasting GIP, and glucose-stimulated GLP-1 and GIP concentrations, between the initial and follow-up studies. The absence of a relationship with fasting GLP-1 may well reflect a type 2 error. Accordingly, despite the substantial interindividual variation in fasting and postprandial GLP-1 and GIP, within the same individual, there is a reproducible pattern. The determinants of this phenomenon remain to be characterised. – The observed slowing of gastric emptying, which may be a determinant of, as well as determined by, plasma GLP-1 concentrations, did not appear to be relevant.

Limitations of our study should be appreciated. The size of the cohort was relatively small and just under 50% of those studied originally participated in the follow-up study with the inherent potential for selection bias. In addition, the 5 subjects with T2D were excluded. Gastric emptying data of another 6 subjects were removed from the analyses due to technical issues, which may have reduced the predictive power of our multiple linear regression models. Furthermore, gastric emptying was assessed by the indirect breath test technique. This method has been shown to correlate closely with scintigraphy, the ‘gold standard’ technique [337, 338],

although the measurements should be regarded as notional, rather than precise [7]. We used the same assays, under the same conditions, to measure GLP-1 and GIP at the initial study and follow-up, but it should be appreciated that inevitably the batch numbers differed given the timing of assays.

In conclusion, our study demonstrates that in healthy older people fasting GLP-1 and GIP, and glucose-stimulated GLP-1 decrease over a period of ~5.9 years, and that longitudinal intra-individual fasting GIP and glucose-stimulated GLP-1 and GIP concentrations correlate. The reduction in incretin hormone responses with ageing may potentially predispose to the development of glucose intolerance and T2D.

Chapter 10

The relationship between plasma glucose-dependent insulinotropic peptide and glucagon-like peptide-1 levels in people with normal and impaired glucose tolerance

Statement of Authorship

Title of paper	The relationship between plasma glucose-dependent insulinotropic peptide and glucagon-like peptide-1 levels in people with normal and impaired glucose tolerance.
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Principle Author

Name of Principal Author (Candidate)	Hung T Pham
Contribution to the Paper	Conducted research, analysed and interpreted data and reviewed paper.
Overall percentage	45%
Certification	This paper reports on original research I conducted during the period of my Higher Degree by Research candidature and is not subject to any obligations or contractual agreements with a third party that would constrain its inclusion in this thesis. I am the joint primary author (equal contribution by CSM and HTP) of this paper.

Signature		Date	Aug 2019

Co-Author Contributions

By signing the Statement of Authorship, each author certifies that:

i. the candidate's stated contribution to the publication is accurate (as detailed above);

ii. permission is granted for the candidate in include the publication in the thesis; and

iii. the sum of all co-author contributions is equal to 100% less the candidate's stated contribution.

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Contribution to the Paper	Conceived and designed research, interpreted data and reviewed paper.		
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Signature		Date	Aug 2019

10.1 Introduction

Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), are released from the small intestine in response to macronutrient exposure (carbohydrate, lipid or protein). GIP is released from enteroendocrine 'K' cells, located predominantly in the proximal small intestine, and GLP-1 from 'L' cells, located predominantly in the distal small intestine and colon [486]. It has been suggested that GIP and GLP-1 account, in approximately equal proportions, for the 'incretin effect' (the amplified insulin secretory response to oral compared with intravenous glucose) in health, although the outcome of a recent study is indicative of a dominant contribution of GIP [487]. The incretin effect is a major determinant of the postprandial insulin secretory response in health, although its magnitude is reduced in type 2 diabetes (T2D), at least in part because the insulinotropic effect of GIP is markedly diminished [290]. GLP-1, unlike GIP, largely retains its insulinotropic (and glucagonostatic) properties in T2D, which has stimulated the development, and current widespread use, of GLP-1 based therapy (GLP-1 receptor agonists and DPP-4 inhibitors) for T2D. It is uncertain whether GLP-1 and GIP secretion are related. A study by Nauck et al in first-degree relatives of people with T2D and healthy controls reported a strong correlation between plasma GIP and GLP-1 levels following a 75g oral glucose tolerance test [465, 488], but the number of subjects (n = 15 relatives, n = 10 controls) was small. In general, macronutrients will be exposed earlier to 'K' than 'L' cells, but, unlike rodents, it is not clear if GIP influences GLP-1 secretion [465]. The current study was conducted to evaluate whether there is a relationship between plasma GIP and GLP-1 levels in a larger cohort of individuals with normal (NGT), or impaired (IGT), glucose tolerance.

10.2 Materials and Methods

10.2.1 Participants

100 healthy Caucasian volunteers were recruited through local advertisement. Participants with a history of gastrointestinal disease, other significant medical illness, or taking medication known to affect gastrointestinal motility, were excluded. The Royal Adelaide Hospital Human Research Ethics Committee approved the study protocol, which conformed to the principles of the Declaration of Helsinki, and all subjects provided written, informed consent before their participation.

10.2.2 Protocol

Each subject fasted overnight (14 hours for solids and 12 hours for liquids) and attended the Department of Nuclear Medicine, PET and Bone Densitometry (Royal Adelaide Hospital) at 0800h when they drank 350 mL water containing 75g glucose within 2 minutes. Time zero (t = 0) was considered as the end of consumption of the drink.

10.2.3 Measurements of blood glucose, serum insulin, GIP and GLP-1

Venous blood was obtained at t = -2, 30, 60, 120, 180 and 240 min. Plasma was separated by centrifugation and stored at −70°C for subsequent assays. Blood glucose concentrations were determined using a glucometer (Medisense Precision QID; Abbott Laboratories, Bedford, MA, USA). Each subject was classified, according to WHO criteria, as having NGT (fasting blood glucose < 6.1 mmol/L, and 2 h < 7.8 mmol/L), IGT (2 h blood glucose < 11.1 mmol/L, but > 7.8 mmol/L), or diabetes (fasting blood glucose ≥7.0 mmol/L and/or 2 h blood glucose ≥11.1 mmol/L) [474]. Serum insulin concentrations were determined by ELISA (10-1113 Mercodia, Uppsala, Sweden), with assay sensitivity of 1.0 mU/L and coefficient of variation 2.5% within

assays and 7.4% between assays [299]. Total GLP-1 was measured by radioimmunoassay (GLPIT- 36HK, Millipore, Billerica, MA, USA). The minimum detectable limit was 3 pmol/L, intra and interassay CVs were 7.7% and 9.4%, respectively [299]. Plasma total GIP was measured by radioimmunoassay. The minimum detectable limit was 2 pmol/L, interassay CV was 8.7%, and intraassay CV was 5.0% [299].

10.2.4 Statistical analysis

Blood glucose, serum insulin, plasma GIP and GLP-1 were analysed and presented as changes from baseline (t = -2 min). One-way repeated-measures ANOVA was used to evaluate the effect of time on blood glucose, insulin, GIP and GLP-1. Incremental areas under the curve ($iAUC_{0\text{-}240min}$) for blood glucose, insulin, GIP and GLP-1 were determined for the NGT and IGT groups and compared using Student's paired t-test. Pearson's correlation was used to assess linear relationships between variables. Data are shown as mean values ± SEM. Statistical significance was set at $P < 0.05$.

10.3 Results

All subjects tolerated the study well and there were no adverse events. Nine participants were found to have diabetes and were excluded from the analysis. Of the remaining 91 healthy Caucasian volunteers (46 male and 45 female participants, mean age 68 ± 0.8 years, mean BMI 26 ± 0.3 kg/m^2), 50 had NGT and 41 had IGT. The overall glycaemic response, as assessed by $iAUC_{0\text{-}240\ min}$ for glucose was greater in IGT (310 ± 22 for NGT vs. 630 ± 29 for IGT, $P < 0.0001$, Figure 10.1A).

There were substantial rises in serum insulin, plasma GIP and GLP-1 following the glucose load ($P < 0.01$ for all). The overall insulinaemic response as assessed by $iAUC_{0\text{-}240\ min}$ for insulin was comparable in the two groups (NGT: 8127 ± 917 vs. IGT: 8637 ± 863 mU/L.min, P = NS, Figure 10.1B). There was also no difference in the overall GIP response, as assessed by $iAUC_{0\text{-}240\ min}$ for GIP (NGT: 6027 ± 346 vs. IGT: 5172 ± 371 pmol/L.min; P = NS, Figure 10.1C). In both cohorts, plasma GIP levels were low at baseline, rising promptly following consumption of glucose drink with peak levels at ~t = 30 min, maintained until ~t = 120 min and followed by decline to near baseline level at t = 240 min (Figure 10.1D). The GLP-1 response as assessed by $iAUC_{0\text{-}240\ min}$ for GLP-1 was higher in NGT, but this difference was not significant (NGT: 1550 ± 260 vs. IGT: 1187 ± 176 pmol/L.min, P = NS) (Fig 1C). In both cohorts, plasma GLP-1 levels were low at baseline, and rose promptly following consumption of glucose drink with peak levels at ~t = 30 min, followed by a sharp decline to the baseline level at t = 120 min, which was maintained until t = 240 min. There was no relationship between baseline GIP and GLP-1 in the combined group (R = 0.14, P = NS), NGT (R = 0.13, P = NS) or IGT (R = 0.16, P = NS). There was a significant relationship between the $iAUC_{0\text{-}240\ min}$ for GIP and GLP-1 in the combined group (R = 0.23, P = 0.015), and in the IGT group (R = 0.34, P = 0.01), but not in the NGT group (R = 0.15, P = NS) (Figure 10.2).

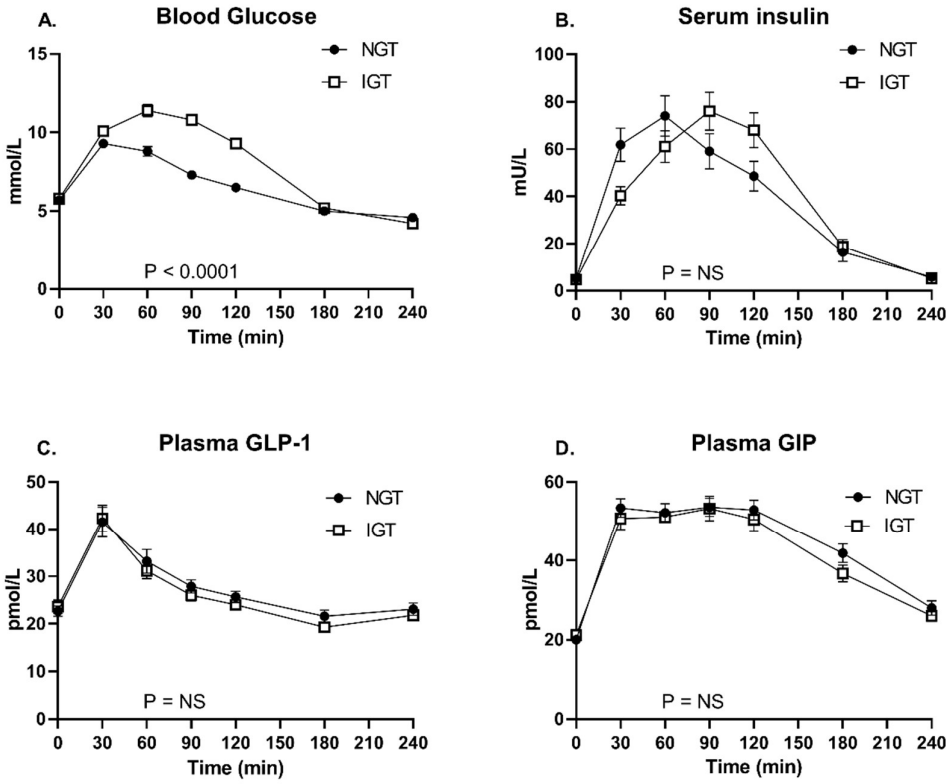

Figure 10.1. Blood glucose (A), serum insulin (B), plasma GLP-1 (C), and plasma GIP (D) levels immediately before and following a 75g oral glucose load in individuals with NGT (n = 50) and those with IGT (n = 41). Data are mean ± SEM.

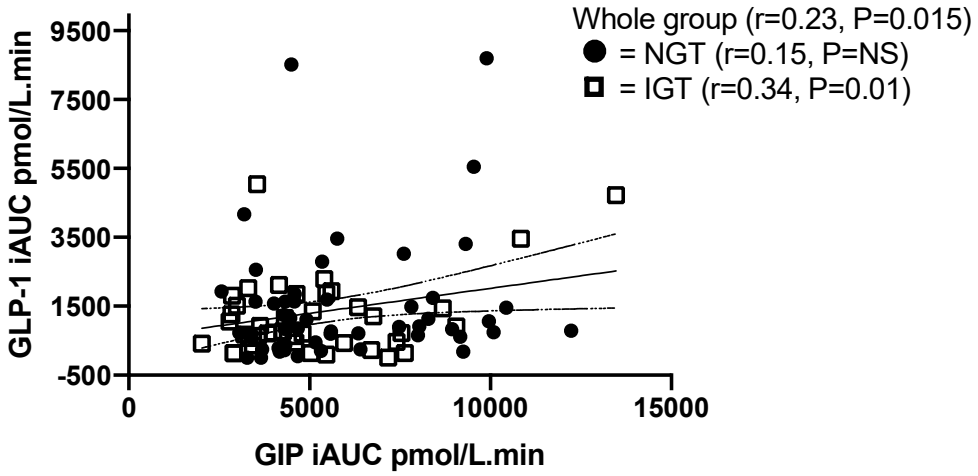

Figure 10.2. Relationship between the incremental areas under the curve (iAUC) from 0-240 min for plasma GIP and GLP-1 in a cohort of people with NGT (n = 50) and IGT (n = 41) following a 75g glucose drink.

10.4 Discussion

We have evaluated, in subjects with (i) NGT or (ii) IGT, whether there is a relationship between the GIP and GLP-1 responses to a 75g oral glucose tolerance test (OGTT). Our observations indicate that there is a relationship which is significant, but only weakly positive. The lack of statistical significance in the NGT group alone may well be a type II error. The factors potentially affecting GLP-1 and GIP secretion following macronutrient ingestion include the type of macronutrient stimulus (e.g. carbohydrate and lipid appear to be more potent stimuli of GLP-1 secretion than protein), caloric content, the rate of delivery of nutrients from the stomach to the small intestine (i.e. gastric emptying), integrity (or the lack of) upper gastrointestinal anatomy (for example, GLP-1 secretion is markedly enhanced post-Roux-en-Y gastric bypass surgery) and the gastrointestinal autonomic system [465]. An association between GIP and GLP-1 levels (and presumably secretion) might reflect (i) a common stimulus or (ii) the influence of one on the other. While GIP and GLP-1 are known to be released from distinct intestinal cells, some entero-endocrine cells may co-secrete GIP and GLP-1 [489]. GIP and GLP-1 share some key commonalities (the same macronutrient stimuli induce the secretion of GIP and GLP-1 and the same ubiquitous enzyme, dipeptidyl peptidase-4 (DPP-4), is responsible for the degradation of both [474]. Intuitively, as K cells are located more proximally, GIP may influence GLP-1 secretion, which is the case in some animal species [490]. However, intravenous infusion of supra-physiological doses of exogenous GIP, does not stimulate GLP-1 secretion in humans [288]).

Our analysis is indicative of only a modest correlation between the secretory responses of the two hormones, which was weaker than that observed by Nauck et al. [488] in a small cohort of first-degree relatives of people with T2D and healthy controls. We did not relate the incretin response to the rate of gastric emptying, which may be a significant determinant [308]. Gastric

emptying exhibits a wide inter-individual variation (between 1-4 kcal/min in health) and our previous studies employing a naso-duodenal catheter to infuse glucose directly into the proximal duodenum have shown that the rate of intraduodenal (ID) delivery of glucose has a major, but differential, impact on both GIP and GLP-1 secretion [308]. When the rate was increased from 1 to 4 kcal/min (i.e. within two extremes of the physiological range of gastric emptying), GIP secretion increased proportionately. In contrast, there was minimal, if any elevation in GLP-1 at rates of 1 to 2 kcal/min, but a sustained and exaggerated response when the rate was increased to 3 kcal/min and beyond, indicative of a 'threshold' rate of delivery (or gastric emptying rate) between 2 and 3 kcal/min [306]. It is, accordingly, possible that a stronger correlation between GIP and GLP-1 might be observed in those individuals who have an intrinsically higher rate of gastric emptying (i.e. >2 kcal/min).

In interpreting our observations, some limitations should be appreciated: (1) a correlation between GIP and GLP-1 secretion does not establish causality; (2) our cohort was comprised predominantly of older men, and it is not known if age affects the relationship; (3) we did not account for variations in gastric emptying or small intestinal absorption; (4) we only characterised the response to glucose and not protein or fat; (5) for convenience, we used a glucometer to determine glucose concentrations, which were taken from venous rather than capillary or arterial samples.

In conclusion, our study indicates that while there is no relationship between fasting GIP and GLP-1, there is a weak, but statistically significant relationship between GIP and GLP-1 responses to oral glucose in individuals without diabetes.

Chapter 11

CONCLUSIONS

This thesis presented a series of studies that provide novel and important insights into the underlying pathophysiology, natural history, and approaches to management, of postprandial hypotension (PPH), as well as longitudinal changes in the pre- and post-prandial release of the incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose dependent insulinotropic polypeptide (GIP) as well as the relationship between them.

In **Chapter 4**, longitudinal changes in the blood pressure (BP) response to, and gastric emptying of, oral glucose in healthy older people were evaluated. After a period of ~5.8 years, the prevalence of PPH doubled from 9.1% to 18.2%. More importantly, gastric emptying was slower and the hypotensive response to glucose, greater, at follow-up. Consistent with previous studies, the fall in systolic BP (SBP) was related directly to the rate of gastric emptying at both the initial study and at follow-up. The change in the maximum fall in SBP was related to the increase in baseline SBP. These observations demonstrated that in healthy older people over a period of ~5.8yr, there was an increased prevalence of PPH and a modest slowing of gastric emptying. The latter was related directly to a greater hypotensive response.

In **Chapter 5,** the acute effects of nutritive and non-nutritive sweeteners on postprandial BP were reported in a systematic review of 62 relevant papers. Current data indicate that the BP response to ingestion of sweeteners is generally unaffected in healthy young subjects, however, in elderly individuals, glucose induces the greatest decrease in postprandial BP, while the response to sucrose is less pronounced. The limited studies investigating other nutritive and non-nutritive sweeteners have demonstrated minimal or no effect on postprandial BP. Dietary modification by replacing high nutritive sweeteners (glucose, fructose, and sucrose) with low nutritive (D-xylose, xylitol, erythritol, maltose, maltodextrin, and tagatose) and non-nutritive sweeteners may be a simple and effective approach in the management of PPH.

Chapter 6 presented the effects of intraduodenal (ID) administration of a widely used artificial sweetener, sucralose, in comparison to saline and glucose, on BP and superior mesenteric artery (SMA) blood flow in healthy older subjects. As expected, blood glucose concentrations increased in response to glucose, but not saline or sucralose. There was a fall in mean arterial BP during ID glucose, but not during ID saline or ID sucralose. Heart rate (HR) and SMA blood flow also increased during ID glucose, but not during ID saline or ID sucralose. Accordingly, ID administration of the artificial sweetener, sucralose, is not associated with changes in BP or SMA blood flow in healthy older subjects. Artificial sweeteners may have therapeutic benefit in the non-pharmacological management of PPH. Further studies are now warranted in these patients.

In **Chapter 7**, the hypothesis that gastric distension (using a water preload administered 15min prior to a test drink) and the α-glucosidase inhibitor, acarbose, may attenuate the postprandial fall in BP by complementary mechanisms was evaluated. The $AUC_{0\text{-}120min}$ for mean arterial pressure (MAP) was greater, and the maximum fall in MAP less, during treatments with acarbose. However, gastric distension did not affect the MAP response to acarbose, and there was no effect of gastric distension alone. Both acarbose treatments attenuated the rise in SMA blood flow, whereas gastric distension had no effect. The study demonstrated that acarbose (100mg), but not gastric distension, attenuates the fall in BP and rise in SMA blood flow after oral sucrose in healthy older adults. These observations support the use of acarbose, but not combined with gastric distension, to attenuate a postprandial fall in BP. However, the effect of a larger volume of water given immediately prior to food ingestion should be investigated further.

Chapter 8 presented the results of a study evaluating the effects of a guar/whey preload given 15 min before a test drink containing 50g glucose in healthy older subjects. Gastric emptying (measured with scintigraphy), intestinal glucose absorption and the glycaemic and BP responses to the glucose drink with or without the guar/whey preload was assessed. The guar/whey protein preload reduced plasma glucose, serum 3-O-methylglucose (3-OMG) and increased plasma insulin. Furthermore, SMA blood flow was reduced and HR increased by the preload. However, there was no difference in gastric emptying or BP between the two days. The reduction in plasma glucose on the preload day compared to control was related to the reduction in glucose absorption. These observations indicate that in healthy older people, the glucose-lowering effect of a whey protein/guar preload may relate to delayed small intestinal glucose absorption and insulin stimulation, rather than slowing of gastric emptying and that the preload has no potential benefit in the management of PPH.

In **Chapter 9**, the first longitudinal assessment of fasting and oral glucose-stimulated incretin hormone responses in a cohort of 41 healthy older people was conducted. Participants had measurements of plasma GLP-1 and GIP while fasting and after a 75g oral glucose load on two occasions separated by 5.9 ± 0.1 years. Breath samples were also collected to calculate the gastric 50% emptying time (T50). For GLP-1, both fasting concentrations and $AUC_{0\text{-}120min}$ were decreased at follow-up. Fasting GIP was also lower at follow-up, but there was no change in the $AUC_{0\text{-}120min}$. The gastric emptying T50 was slower at follow-up. Neither the change in T50 nor the BMI at the initial study was a determinant of the change in incretin responses. Between the two study days, fasting GIP correlated well, but not fasting GLP-1. However, both glucose-stimulated GLP-1 and GIP showed correlations between the initial and follow-up studies. In summary, fasting GIP, and glucose-stimulated GLP-1 and GIP concentrations correlate within individuals over a follow-up period of ~ 5.9 years. Ageing is associated with

reductions in fasting GLP-1 and GIP, and glucose-stimulated GLP-1, which may predispose to the development of glucose intolerance and type 2 diabetes (T2D).

In **Chapter 10**, the relationship between plasma GIP and GLP-1 responses to 75g oral glucose in individuals with normal (NGT), or impaired (IGT), glucose tolerance was reported. In both groups, there were increases in plasma GIP and GLP-1 following the glucose drink, with no difference in the magnitude of the responses between t = 0-240 min. There was a weak relationship between the $iAUC_{0\text{-}240\ min}$ for GIP and GLP-1 in the combined and in the IGT, but not in the NGT group. Our conclusion is that there is a weak relationship between oral glucose-induced GIP and GLP-1 secretion in a cohort of non-diabetic individuals. Further studies are required in T2D.

In summary, while the studies presented in this thesis have provided fundamental insights relating to postprandial BP regulation in healthy older people, future studies are now warranted in patients with symptomatic PPH. The studies relating to GIP and GLP-1 secretion indicate changes with ageing and a correlation between, GLP-1 and GIP. Future studies with larger samples sizes and in patients with T2D are now required.

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